Double **determinant immuno-polymerase** chain **reaction: a sensitive method for detecting circulating antigens in human sera.**

Suzuki A ; Itoh F; Hinoda Y; Imai K

First Department of Internal Medicine, Sapporo Medical University School of Medicine.

Japanese journal of cancer research (JAPAN) Sep 1995, 86 (9) p885-9, ISSN 0910-5050 Journal Code: HBA

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9602

Subfile: INDEX MEDICUS

A sensitive method for the detection of antigens in sera, termed double determinant immunopolymerase chain reaction (double determinant immuno-PCR) was developed, using two monoclonal antibodies (MoAbs), in which the antigens are sandwiched, and a specific DNA molecule is used as a marker. Instead of the antigen itself, the first MoAb to bind the circulating antigens was immobilized. After the biotinylated second MoAb was bound to the antigen, free streptavidin was used to attach a biotinylated DNA to the biotinylated second MoAb. The biotinylated DNA complexed with antigen-antibody-streptavidin was amplified by PCR, and the PCR products were analyzed by Southern blot hybridization after agarose gel electrophoresis. Compared with the conventional enzyme linked immunosorbent assay (ELISA) using soluble intercellular adhesion molecule-1 (sICAM-1) in the supernatant of cultured Panc-1 ' cells as an antigen, our double determinant immuno-PCR was 10(3) times more sensitive in terms of the detection limit. Not only in culture medium, but also in sera from gastric cancer patients of high sICAM-1 titer, an approximately 10(3)-fold enhancement in detection sensitivity was obtained compared with ELISA. In addition, this system can detect the antigen in sera at a level below the detection limit of traditional ELISA methods with high sensitivity. Thus, double determinant immuno-PCR has the significant advantage that it can be readily applied to any antigen-antibody system for which two MoAbs are available.

Tags: Comparative Study; Human; Support, Non-U.S. Gov't

Immuno-PCR: very sensitive antigen detection by means of specific antibody-DNA conjugates.

Sano T ; Smith CL; Cantor CR

Department of Molecular and Cell Biology, University of California, Berkeley 94720.

Science (UNITED STATES) Oct 2 **1992** , 258 (5079) p120-2, ISSN 0036-8075 Journal Code: UJ7

Contract/Grant No.: CA39782, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9302

Subfile: INDEX MEDICUS

An antigen detection system, termed immuno-**polymerase** chain reaction (immuno-PCR), was developed in which a specific DNA molecule is used as the marker. A streptavidin-protein A chimera that possesses tight and specific binding affinity both for biotin and immunoglobulin G was used to attach a biotinylated DNA specifically to antigen-monoclonal antibody complexes that had been immobilized on microtiter plate wells. Then, a segment of the attached DNA was amplified by PCR. Analysis of the PCR products by agarose gel electrophoresis after staining with ethidium bromide allowed as few as 580 antigen molecules (9.6 x 10(-22) moles) to be readily and reproducibly detected. Direct comparison with enzyme-linked immunosorbent assay with the use of a chimera-alkaline phosphatase conjugate demonstrates that enhancement (approximately x 10(5)) in detection sensitivity was obtained with the use of immuno-PCR. Given the enormous amplification capability and specificity of PCR, this immuno-PCR technology has a sensitivity greater than any existing antigen detection system and, in principle, could be applied to the detection of single antigen molecules.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: Antigens--Analysis--AN; * **Polymerase** Chain Reaction --Methods--MT; Antibodies, Monoclonal; Densitometry; DNA; Electrophoresis, Agar Gel; Enzyme-Linked Immunosorbent Assay; Sensitivity and Specificity

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Antigens); 9007-49-2 (DNA)

Transcription-based amplification system and detection of amplified human immunodeficiency virus type 1 with a bead-based sandwich hybridization format.

Kwoh DY ; Davis GR; Whitfield KM; Chappelle HL; DiMichele LJ; Gingeras TR

SISKA Diagnostics, San Diego, CA 92138-9216.

Proc Natl Acad Sci U S A (UNITED STATES) Feb **1989** , 86 (4) p1173-7, ISSN 0027-8424 Journal Code: PV3

Contract/Grant No.: NO1-HB-6-7019, HB, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8906

Subfile: INDEX MEDICUS

The in vitro amplification of biologically important nucleic acids has proceeded principally by a strategy of DNA replication. Polymerase chain reaction was the first such protocol to achieve this goal. In this report, a transcription-based amplification system (TAS) is described. Each cycle of the TAS is composed of two steps. The first is a cDNA synthesis step that produces one copy of a double-stranded DNA template for each copy of RNA or DNA target nucleic acid. During the course of this cDNA synthesis step, a sequence recognized by a DNA-dependent RNA polymerase is inserted into the cDNA copy of the target sequence to be amplified. The second step is the amplification of the target sequence by the transcription of the cDNA template into multiple copies of RNA. This procedure has been applied to the detection of human immunodeficiency virus type 1 (HIV-1)-infected cells. After four cycles of TAS, the amplification of the vif region of the HIV-1 RNA genome was measured to be, on the average, 38- to 47-fold per cycle, resulting in a 2-5 x 10(6)-fold increase in the copy number of the original target sequence. This amplification by the TAS protocol allows the detection of fewer than one HIV-1-infected CEM cell in a population of 10(6) uninfected CEM cells. Detection of the TAS-generated RNA from HIV-1-infected cells can easily be accomplished by means of a bead-based sandwich hybridization protocol, which provides additional specificity for the identification of the amplified HIV-1-specific sequence.

Tags: Human; Support, U.S. Gov't, P.H.S.

Descriptors: *Gene Amplification; *Genes, Viral; *HIV-1--Genetics--GE; *Transcription, Genetic; Cell Line; Cell Transformation, Viral; Nucleic Acid Hybridization; Oligonucleotide Probes; RNA, Viral--Genetics--GE; RNA, Viral--Isolation and Purification--IP

CAS Registry No.: 0 (Oligonucleotide Probes); 0 (RNA, Viral)

US PAT NO: 5,728,530 [IMAGE AVAILABLE] L2: 47 of 153

DETDESC:

DETD(18)

The **Ligase**-Chain-reaction (**LCR**, EP-A-0 320 308) can also be improved in accordance with the invention. Instead of at least one oligonucleotide, this improvement uses a set of oligonucleotides in accordance with the invention. FIG. 13 demonstrates that the number of mismatches in **cross** products is increased. Depending on the location of Y, the extension and/or annealing of incorrect oligonucleotides is largely suppressed. In. . . be located at the 3'-end of the one oligonucleotide or at the 5'-end of the other oligonucleotide. In principle, an **LCR** where X is located the the middle of the oligonucleotide can be carried out analgously to COP process. Preferred are. . .

US PAT NO: 5,605,794 [IMAGE AVAILABLE] L2: 76 of 153

SUMMARY:

BSUM(64)

The **Ligase**-Chain-reaction (**LCR**, EP-A-0 320 308) can also be improved in accordance with the invention. Instead of at least one oligonucleotide, this improvement uses a set of oligonucleotides in accordance with the invention. FIG. 13 demonstrates that the number of mismatches in **cross** products is increased.

US PAT NO: 5,876,976 [IMAGE AVAILABLE] L2: 6 of 153

DRAWING DESC:

DRWD(4)

FIG. 3 is a diagram showing **cross**-linking agent modification of an **LCR**-derived amplification product and subsequent irreversible **cross**-linking of the modified amplification product.

DETDESC:

DETD(41)

The **cross**-linking agent is incorporated into the amplification probe(s) or primer(s) using methods known to those skilled in the art. In an **LCR** type of amplification procedure, it is preferred to locate the **cross**-linking agent on a middle amplification probe which will, in turn, incorporate the **cross**-linking agent nearest to the center of the resulting amplification product as is practically possible. With respect to the amplification probe carrying the **cross**-linking agent, it is further preferred to locate the **cross**-linking agent in the center region of the probe so as not to interfere with the joining or ligating of the ends of the modified probe. This is demonstrated in FIG. 3, wherein the **cross**-linking modification is incorporated into amplification product from one of the middle pair of probes of a three pair set of. . .

US PAT NO: 5,849,878 [IMAGE AVAILABLE] L2: 16 of 153

DETDESC:

DETD(114)

Ever . . . a non-genetic chemical has yet to be fully realized. This Example shows that end-derivatized double-stranded DNA can serve as a **cross**-linker between different immunoglobulin G Fab' fragments to form a semisynthetic bispecific antibody. Beyond its function as a novel **cross**-linker, though, DNA possesses a number of potentially very useful properties, including the following: gentle hetero-**cross**-linking via simple annealing reactions; automated synthesis; controllable sequence variation resulting in well-defined length and structure (useful up to hundreds of. . . and exquisitely sensitive detection via the polymerase chain reaction. However, to enable enzymatic action by restriction enzymes against the intact **cross**-linking DNA molecule, a somewhat longer DNA sequence (SEQ ID NOS:1 and 2) should be used as the 32mer sequence used. . . steric hindrance or altered DNA structure. Another difficulty is that reducing conditions are necessary for many DNA modifying enzymes, including **ligases** and many restriction enzymes. Unfortunately, the model constructs described in this paper all contain easily usable disulfide bond **cross**-links between protein and DNA. Treatment with reducing agent will destroy these important structural bonds. For applications that require irreversiblity of the protein-DNA **cross**-link, an irreversible thioether bond may be produced (Glennie e

S PAT NO: 5,814,492 [IMAGE AVAILABLE] L2: 32 of 153

DRAWING DESC:

DRWD(8)

FIG. . . . the invention using four blocking oligos (indicated by asterisk "*"), one on each of the four probes used in gap **LCR**. In this case, interference control is provided by a "bubble" deletion and by chemical groups on each of the blocking. . . amplification probes and allowed to hybridize to one another. In a variation of this, the hairpin blocking oligo may be **crosslinked** in the hinge region to prevent "unbending" of the hairpin.

DETDESC:

DETD(23)

A . . . so that the self complementary ends can fold back over one another and anneal. The hairpin end may optionally be **crosslinked** to itself to prevent unfolding. In this configuration it is generally preferable that the 5' terminal base lie adjacent the 3' terminal base of the probe to provide the most efficient mask. However, in **LCR** mixtures containing **ligase** the 5' end of the blocking oligo should not be phosphorylated or it should terminate with at least one base. .

DETDESC:

DETD(24)

The . . . hairpin (a minimum of about 7 or 8 nucleotides). In cases where hybridization sequestration is used as interference control in **LCR** (see below), the hairpin may be relatively long and is preferably complementary to a similar hairpin on the blocking oligo. . . oligo homoduplex, without affecting the Tm of the blocking oligo:probe heteroduplex. Hairpins used in sequestration interference control are preferably not **crosslinked**.

US PAT NO: 5,802,921 [IMAGE AVAILABLE] L2: 33 of 153

DETDESC:

DETD(27)

In the case of a particular **cross** modification that is to be classified on a geometric basis, the criterion is the amount of working surface material the **cross** modification removes at or beyond (i.e., farthest from the field entry or exit corner, as the case may be) the outermost pair of symmetrically arrayed contact lines. Ideally the **crosshatched** areas (92 and 93 in FIG. 9 or 112 and 113 in FIG. 11) will not reach these outermost lines,. . . of the spikeshaped area 112, 113 that overlaps the contact line (e.g., segment r-120 in FIG. 11). To restrict the **cross** modification to a region in which it will not produce detrimental initial separation along any significant portion of these outermost lines of contact, the combined area shown **crosshatched** in fields such as those shown in FIGS. 9 and 11 should not exceed a certain critical percentage of the total field area. That critical percentage is different for fields of different proportions,

such as HCR and **LCR** gears, so it is much simpler and more direct to define the limits of liminal **cross** modification by reference to the base pitch.

US PAT NO: 5,780,231 [IMAGE AVAILABLE] L2: 38 of 153

DETDESC:

DETD(11)

In one aspect, the oligonucleotide tags of the invention comprise a plurality of "words" or subunits selected from minimally **cross**-hybridizing sets of subunits. Subunits of such sets cannot form a duplex or triplex with the complement of another subunit of. . . embodiments, sequences of any two oligonucleotide tags of a repertoire can be even "further" apart, e.g. by designing a minimally **cross**-hybridizing set such that subunits cannot form a duplex with the complement of another subunit of the same set with less. . . and their complements are oligomers of the natural nucleotides so that they may be conveniently processed by enzymes, such as **ligases**, polymerases, nucleases, terminal transferases, and the like.

US PAT NO: 5,763,175 [IMAGE AVAILABLE] L2: 45 of 153

DETDESC:

DETD(8)

In one aspect, the oligonucleotide tags of the invention comprise a plurality of "words" or subunits selected from minimally **cross**-hybridizing sets of subunits. Subunits of such sets cannot form a duplex or triplex with the complement of another subunit of. . . embodiments, sequences of any two oligonucleotide tags of a repertoire can be even "further" apart, e.g. by designing a minimally **cross**-hybridizing set such that subunits cannot form a duplex with the complement of another subunit of the same set with less. . . and their complements are oligomers of the natural nucleotides so that they may be conveniently processed by enzymes, such as **ligases**, polymerases, nucleases, terminal transferases, and the like.

and other genomes is accumulating rapidly. However, it frequently proves difficult to use such short DNA segments to identify clones in genomic libraries or fragments in blots of the whole genome or for in situ analysis of chromosomes. Oligonucleotide probes, consisting of two target-complementary segments, connected by a linker sequence, were designed. Upon recognition of the specific nucleic acid molecule the ends of the probes were joined through the action of a %ligase%, creating circular DNA molecules catenated to the target sequence. These probes thus provide highly specific detection with minimal background.

7/7/2 (Item 2 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

11203473 BIOSIS Number: 97403473
Solid-phase synthesis of chelate-labelled oligonucleotides: Application in triple-color %ligase%-mediated gene analysis
Kwiatkowski M; Samiotaki M; Lamminmaki U; Mukkala V-M; %Landegren U%
Beijer Lab., Dep. Med. Genetics, Box 589 BMC, S-75123 Uppsala, SWE
Nucleic Acids Research 22 (13). 1994. 2604-2611.
Full Journal Title: Nucleic Acids Research
ISSN: 0305-1048
Language: ENGLISH
Print Number: Biological Abstracts Vol. 098 Iss. 006 Ref. 074285
Oligonucleotides labelled with detectable groups are essential tools in gene detection. We describe here the synthesis of pyrimidine deoxynucleotide-building blocks, modified at their C-5 position with a protected form of a strongly chelating agent. These reagents can be used to introduce multiple metal ions into oligodeoxynucleotides during standard oligonucleotide synthesis. The chelating functions form strongly fluorescent complexes with europium ions, characterized by a wide separation between the excitation and emission spectra. Moreover, the long decay time of the fluorescence permits sensitive time-resolved fluorescence measurements. The chelates also have the stability required to function in triple-color assays involving europium, samarium, and terbium ions. We demonstrate the application of these reagents for %ligase%-based gene analysis reactions.

7/7/3 (Item 3 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

11023511 BIOSIS Number: 97223511
Dual-color detection of DNA sequence variants by %ligase%-mediated analysis
Samiotaki M; Kwiatkowski M; Parik J; %Landegren U%
Dep. Med. Genetics, Box 589, Uppsala Biomed. Cent., S-75123 Uppsala, SWE
Genomics 20 (2). 1994. 238-242.
Full Journal Title: Genomics
ISSN: 0888-7543
Language: ENGLISH
Print Number: Biological Abstracts Vol. 097 Iss. 010 Ref. 140626
Genetic screening for sequence variants associated with disease is assuming increasing importance in clinical medicine as well as in research. We describe an efficient method for such analyses, comprising a combination of practical features: (1) Amplified DNA samples are analyzed for their ability to serve as templates in standardized allele-specific %ligation%

res. In FIG. 3 AbDSD is an antibody to double stranded DNA, and in FIG. 4 AbD is anti-digoxigenin antibody and D is digoxigenin. Hybridisation techniques using these methodologies are already known, see for example:- Guesdon J-L (1992), "Immunoenzymatic Techniques Applied to the Specific Detection of Nucleic Acids", Journal of Immunological Methods 150, 33-49; Mantero G, Zonaro A, Albertini A, Bertolo P & Primi D. (1991), "DNA Enzyme **Immunoassay**: General Method for Detecting Products of **Polymerase Chain Reaction**", Clinical Chemistry 37/3, 422-429; Keller G. H., Huang D-P, Shih W-K & Manak M. M. (1990), "Detection of Hepatitis B Virus DNA in Serum by **Polymerase Chain Reaction** Amplification and Microtiter Sandwich Hybridization", Journal of Clinical Microbiology 28/6, 1411-1416; Nickerson D. A., Kaiser R., Lappin S, Stewart J, Hood L & Landegren U (1990), "Automated DNA Diagnostics Using an ELISA-based Oligonucleotide **Ligation** Assay", Proceedings of the National Academy of Sciences 87, 8923-8927; Wolf S. F., Haines L., Fisch J., Kremsky J. N., Dougherty J. P. & Jacobs K. (1987), "Rapid Hybridization Kinetics of DNA Attached to Submicron Latex Particles", Nucleic Acids Research 15/7, 2911-2926.

1 2 3 4 5

12, 10, 9, 8, 6, 5, 3, 1, 4

4,988,617

15,

9416105

Oligonucleotide ligation assay for detecting mutations in the human immunodeficiency virus type 1 pol gene that are associated with resistance to zidovudine, didanosine and lamivudine

Edelstein R E; **Nickerson D A**; Tobe V O; Manns-Arcuino L A; Frenkel L M

4800 Sand Point Way NE, CH-32, Seattle, WA 98105, USA

Journal of Clinical Microbiology 36 (2). 1998. 569-572.

Full Journal Title: Journal of Clinical Microbiology

ISSN: 0095-1137

Language: ENGLISH

Print Number: Biological Abstracts Vol. 105 Iss. 006 Ref. 079453

This report describes the detection of mutations in the pol gene of human immunodeficiency virus type 1 associated with resistance to zidovudine, didanosine, and lamivudine by genotyping by an **oligonucleotide ligation** assay specific codons in the pol gene amplified by PCR. Our studies demonstrate the sensitivity, simplicity, and specificity of this genotyping system.

Descriptors/Keywords: RESEARCH ARTICLE; HUMAN IMMUNODEFICIENCY VIRUS TYPE 1; HIV-1; PATHOGEN; POL GENE; MUTATION; ZIDOVUDINE; ANTIVIRAL-DRUG; DIDANOSINE; ANTIVIRAL-DRUG; LAMIVUDINE; ANTIVIRAL-DRUG; **OLIGONUCLEOTIDE LIGATION** ASSAY; ANTIVIRAL RESISTANCE; MOLECULAR GENETICS; PCR; POLYMERASE CHAIN REACTION; METHODOLOGY; NUCLEOTIDE SEQUENCE; ANALYTICAL METHOD; GENETIC METHOD; AMPLIFICATION METHOD

Concept Codes:

*10504 Biophysics-General Biophysical Techniques
*10506 Biophysics-Molecular Properties and Macromolecules
*31500 Genetics of Bacteria and Viruses
*33506 Virology-Animal Host Viruses
*36006 Medical and Clinical Microbiology-Virology
*38506 Chemotherapy-Antiviral Agents
10060 Biochemical Studies-General

Biosystematic Codes:

02623 Retroviridae (1993-)

Super Taxa:

Microorganisms; Viruses

5/9/2 (Item 2 from file: 5)

DIALOG(R)File 5:BIOSIS PREVIEWS(R)

13260123 BIOSIS Number: 99260123

Single-well genotyping of diallelic sequence variations by a two-color ELISA-based **oligonucleotide ligation** assay

Tobe V O; Taylor S L; **Nickerson D A**

Dep. Mol. Biotechnol., Box 357730, Univ. Washington, Seattle, WA 98195-7730, USA

Nucleic Acids Research 24 (19). 1996. 3728-3732.

Full Journal Title: Nucleic Acids Research

ISSN: 0305-1048

Language: ENGLISH

Print Number: Biological Abstracts Vol. 102 Iss. 012 Ref. 175753

Single nucleotide substitutions and unique insertions/ deletions are the most common form of DNA sequence variation and disease-causing mutation in the human genome. Because of the biological and medical importance of these variations, a wide array of methods have been developed for their typing. We have applied an approach that combines the amplification of polymorphic regions by the polymerase chain reaction (PCR) with a system for typing

diallelic variants using an **oligonucleotide ligation** assay (OLA). In this report, we describe a significant advance in this technology that permits the typing of two alleles in a single microtiter well. By marking each of the allele-specific primers with a unique hapten, i.e. digoxigenin and fluorescein, each OLA reaction can be detected by using hapten specific antibodies that are labeled with different enzyme reporters, alkaline phosphatase or horseradish peroxidase. This system permits the detection of the two alleles using a high throughput format that leads to the production of two different colors. We demonstrate the specificity, sensitivity and ease of data interpretation with this system. Furthermore, we show that multiplex PCR/OLA not only increases the throughput of DNA typing but also increases its accuracy in typing diallelic sequence variations using an approach that can be broadly applied for human genome analysis (in evaluating genotype/phenotype links), in typing infectious agents and in forensic analysis.

Descriptors/Keywords: RESEARCH ARTICLE; HUMAN; DIALLELIC SEQUENCE VARIATIONS; TWO-COLOR ELISA-BASED **OLIGONUCLEOTIDE LIGATION** ASSAY; GENOME ANALYSIS; MOLECULAR GENETICS; METHODOLOGY; GENOTYPING; ANALYTICAL METHOD; SINGLE-WELL GENOTYPING METHOD

Concept Codes:

*03502 Genetics and Cytogenetics-General
*03508 Genetics and Cytogenetics-Human
*10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines
*10504 Biophysics-General Biophysical Techniques
*10804 Enzymes-Methods
*34508 Immunology and Immunochemistry-Immunopathology, Tissue Immunology

Biosystematic Codes:

86215 Hominidae

Super Taxa:

Animals; Chordates; Vertebrates; Mammals; Primates; Humans

5/9/3 (Item 3 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

13038836 BIOSIS Number: 99038836

Testing the feasibility of DNA typing for human identification by PCR and an **oligonucleotide ligation** assay

Delahunty C; Ankener W; Deng Q; Eng J; **Nickerson D A**
Dep. Molecular Biotechnology, Box 357730, Univ. Washington, Seattle, WA 98195-7730, USA

American Journal of Human Genetics 58 (6). 1996. 1239-1246.
Full Journal Title: American Journal of Human Genetics
ISSN: 0002-9297
Language: ENGLISH
Print Number: Biological Abstracts Vol. 102 Iss. 002 Ref. 021009

The use of DNA typing in human genome analysis is increasing and finding widespread application in the area of forensic and paternity testing. In this report, we explore the feasibility of typing single nucleotide polymorphisms (SNPs) by using a semiautomated method for analyzing human DNA samples. In this approach, PCR is used to amplify segments of human DNA containing a common SNP. Allelic nucleotides in the amplified product are then typed by a colorimetric implementation of the **oligonucleotide ligation** assay (OLA). The results of the combined assay, PCR/OLA, are read directly by a spectrophotometer; the absorbances are compiled; and the genotypes are automatically determined. A panel of 20 markers has been developed for DNA typing and has been tested using a sample panel from the

CEPH pedigrees (CEPH parents). The results of this typing, as well as the potential to apply this method to larger populations, are discussed.
Descriptors/Keywords: RESEARCH ARTICLE; HUMAN; NUCLEOTIDE SEQUENCE; MOLECULAR SEQUENCE DATA; FORENSICS; POLYMERASE CHAIN REACTION; ANALYTICAL METHOD
Concept Codes:
*00531 General Biology-Forensic Science
*03508 Genetics and Cytogenetics-Human
*10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines
*10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
*10804 Enzymes-Methods
Biosystematic Codes:
86215 Hominidae
Super Taxa:
Animals; Chordates; Vertebrates; Mammals; Primates; Humans

5/9/4 (Item 4 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

11530415 BIOSIS Number: 98130415
Finnish-type aspartylglucosaminuria detected by **oligonucleotide ligation** assay
Delahunty C M; Ankener W; Brainerd S; **Nickerson D A**; Mononen I T
Dep. Molecular Biotechnology, Univ. Washington, Seattle, WA 98195, USA
Clinical Chemistry 41 (1). 1995. 59-61.
Full Journal Title: Clinical Chemistry
ISSN: 0009-9147
Language: ENGLISH
Print Number: Biological Abstracts Vol. 099 Iss. 006 Ref. 086972
Aspartylglycosaminuria (AGU) is a recessively inherited lysosomal storage disease that occurs with much higher frequency in Finland than elsewhere. AGU is caused by a deficiency in glycosylasparaginase (GA), which results in the accumulation of glycoasparagines in lysosomes. In the Finnish population, a single nucleotide change in the gene encoding GA is responsible for the disease. We have used the **oligonucleotide ligation** assay (OLA) to detect the mutation in polymerase chain reaction (PCR) amplified DNA samples from normal, carrier, and affected individuals. Screening for AGU among 415 random Finnish DNA samples with PCR/OLA revealed five carriers of the mutant allele and demonstrated the potential of the method for use in carrier screening. PCR/OLA provides a rapid, reliable, nonisotopic method to detect the mutation responsible for AGU that can readily be applied to large population screening.
Descriptors/Keywords: RESEARCH ARTICLE; HUMAN; GLYCOSYLASPARAGINASE DEFICIENCY; LYSOSOMAL STORAGE DISEASE; GENETIC DISORDER; POLYMERASE CHAIN REACTION; PSYCHOMOTOR RETARDATION; EPIDEMIOLOGY; DIAGNOSTIC SCREENING; FINLAND
Concept Codes:
*03508 Genetics and Cytogenetics-Human
*10006 Clinical Biochemistry; General Methods and Applications
*10804 Enzymes-Methods
*10808 Enzymes-Physiological Studies
*12504 Pathology, General and Miscellaneous-Diagnostic
*13012 Metabolism-Proteins, Peptides and Amino Acids
*13020 Metabolism-Metabolic Disorders
*20506 Nervous System-Pathology
*21006 Psychiatry-Mental Retardation
*37054 Public Health: Epidemiology-Organic Diseases and Neoplasms

07004 Behavioral Biology-Human Behavior
10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines
10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
Biosystematic Codes:
86215 Hominidae
Super Taxa:
Animals; Chordates; Vertebrates; Mammals; Primates; Humans

5/9/5 (Item 5 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

9598783 BIOSIS Number: 94103783
AUTOMATABLE SCREENING OF YEAST ARTIFICIAL-CHROMOSOME LIBRARIES BASED ON THE **OLIGONUCLEOTIDE-LIGATION** ASSAY
KWOK P-Y; GREMAUD M F; **NICKERSON D A**; HOOD L; OLSON M V
DEP. GENETICS, WASHINGTON UNIVERSITY SCHOOL MEDICINE, ST. LOUIS, MO. 63110.
GENOMICS 13 (4). 1992. 935-941. CODEN: GNMCE
Full Journal Title: Genomics
Language: ENGLISH
The systematic screening of yeast artificial-chromosome (YAC) libraries is the limiting step in many physical mapping projects. To improve the screening throughput for a human YAC library, we designed an automatable strategy to identify YAC clones containing a specific segment of DNA. Our approach combines amplification of the target sequence from pooled YAC DNA by the polymerase chain reaction (PCR) with detection of the sequence by an ELISA-based **oligonucleotide-ligation** assay (OLA). The PCR-OLA approach eliminates the use of radioactive isotopes and gel electrophoresis, two of the major obstacles to automated YAC screening. Furthermore, the use of the OLA to test for the presence of sequences internal to PCR primers provides an additional level of sensitivity and specificity in comparison to methods that rely solely on the PCR.
Descriptors/Keywords: HUMAN POLYMERASE CHAIN REACTION METHOD NUCLEOTIDE SEQUENCE MOLECULAR SEQUENCE DATA
Concept Codes:
*03504 Genetics and Cytogenetics-Plant
*03508 Genetics and Cytogenetics-Human
*10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines
*10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
Biosystematic Codes:
15000 Fungi-Unspecified
86215 Hominidae
Super Taxa:
Microorganisms; Plants; Nonvascular Plants; Fungi; Animals; Chordates; Vertebrates; Mammals; Primates; Humans

5/9/6 (Item 6 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

9091738 BIOSIS Number: 93076738
IDENTIFICATION OF CLUSTERS OF BIALLELIC POLYMORPHIC SEQUENCE-TAGGED SITES PSTSS THAT GENERATE HIGHLY INFORMATIVE AND AUTOMATABLE MARKERS FOR GENETIC LINKAGE MAPPING
NICKERSON D A; WHITEHURST C; BOYSEN C; CHARMLEY P; KAISER R; HOOD L

DIV. BIOL., 139-74, CALIF. INST. TECHNOL., PASADENA, CALIF. 91125.
GENOMICS 12 (2). 1992. 377-387. CODEN: GNMCE
Full Journal Title: Genomics
Language: ENGLISH

Using a combination of denaturing gradient gel electrophoresis and direct DNA sequencing, we have found that multiple (4 to 7) biallelic sequence polymorphisms can be located within short DNA segments, 300 to 2400 bp. Here, we report on the identification of three clusters of DNA polymorphisms, one in each of the constant regions of the human T cell receptor .alpha. and .beta. gene complexes on human chromosomes 14 and 7, respectively and a third among the human t-RNA genes on human chromosome 14. The frequency of these polymorphisms and the extent of linkage disequilibrium between individual polymorphisms have been determined using a semiautomated DNA typing system combining DNA target amplification by the polymerase chain reaction with the analysis of internal sequence polymorphisms by a colorimetric **oligonucleotide ligation** assay.
We have found that individual biallelic polymorphisms in each cluster are often in partial linkage disequilibrium with one another. This partial linkage disequilibrium permits the combined use of three to four markers in a cluster to generate a halotype with high levels of heterozygosity, 71 to 88%. Therefore, clusters of physically linked biallelic polymorphisms provide an automatable and highly informative type of genetic marker for general linkage analysis as well as an attractive alternative marker system for fine-point mapping of disease-causing genes and phenotypic traits relative to their framework locations in the genome.

Descriptors/Keywords: HUMAN DNA NUCLEOTIDE SEQUENCE MOLECULAR SEQUENCE DATA POLYMERASE CHAIN REACTION DENATURING GRADIENT GEL ELECTROPHORESIS
Concept Codes:
*02508 Cytology and Cytochemistry-Human
*03508 Genetics and Cytogenetics-Human
*10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
*10506 Biophysics-Molecular Properties and Macromolecules
Biosystematic Codes:
86215 Hominidae
Super Taxa:
Animals; Chordates; Vertebrates; Mammals; Primates; Humans

5/9/7 (Item 7 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

8564438 BIOSIS Number: 92029438
ANALYSIS OF MHC CLASS I GENE EXPRESSION IN ADULT BONE MARROW AND FETAL LIVER OF THE BALB-C MOUSE
CHEROUTRE H; KRONENBERG M; BRORSON K; HUNT S W III; EGHTESADY P; HOOD L; **NICKERSON D A**
DEP. MICROBIOL. IMMUNOL., UCLA CENT. HEALTH SCI., 10833 LE CONTE AVE., LOS ANGELES, CALIF. 90024-1747.
J IMMUNOL 146 (10). 1991. 3263-3272. CODEN: JOIMA
Full Journal Title: Journal of Immunology
Language: ENGLISH

The BALB/c mouse has at least 29 class I genes encoded in the Qa, Tla, and Hmt (histocompatibility dependent on the maternally transmitted factor) regions of the MHC. The pattern of expression of these class Ib MHC genes is not well characterized, although some of their products such as the serologically detectable Qa-2 and TL Ag are expressed mainly in lymphoid and hematopoietic tissues. In this study, the expression pattern of BALB/c class I genes has been analyzed in adult bone marrow and fetal liver. cDNA

libraries were synthesized from these tissues, and isolated class I cDNA clones were characterized by hybridization with **oligonucleotide** probes and by nucleotide sequence analysis. Of the 29 total class Ib genes, transcripts of five including Q6d, Q7d, T9c, T10c, and the 37 gene were isolated from the bone marrow cDNA library. Four of these can encode proteins; the sequence of the T10c gene demonstrates it most likely a pseudogene. A nonoverlapping set of three class Ib cDNA clones was obtained from the fetal liver, including T13c, the Thy 19.4 gene, and a previously uncharacterized class I gene provisionally designated as FL 57.2. Although the majority of H-2Dd cDNA clones that were analyzed lack introns, many of the class Ib cDNA clones contain intron sequences. This suggests that the expression of some of these genes may be regulated at the level of RNA splicing. The T13c gene encodes the thymus leukemia Ag in BALB/c mice. We have confirmed that the T13c gene is expressed in fetal liver by flow cytometric analysis of cells stained with anti-TL mAb.
Descriptors/Keywords: T-10C GENE PSEUDOGENE RNA SPLICING MAJOR HISTOCOMPATIBILITY COMPLEX THYMUS LEUKEMIA ANTIGEN
Concept Codes:
*03506 Genetics and Cytogenetics-Animal
*10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
*10064 Biochemical Studies-Proteins, Peptides and Amino Acids
*14004 Digestive System-Physiology and Biochemistry
*15004 Blood, Blood-Forming Organs and Body Fluids-Blood Cell Studies
*15006 Blood, Blood-Forming Organs and Body Fluids-Blood, Lymphatic and Reticuloendothelial Pathologies
*15008 Blood, Blood-Forming Organs and Body Fluids-Lymphatic Tissue and Reticuloendothelial System
*24003 Neoplasms and Neoplastic Agents-Immunology
*24010 Neoplasms and Neoplastic Agents-Blood and Reticuloendothelial Neoplasms
*25502 Developmental Biology-Embryology-General and Descriptive
*34508 Immunology and Immunochemistry-Immunopathology, Tissue Immunology
10068 Biochemical Studies-Carbohydrates
18001 Bones, Joints, Fasciae, Connective and Adipose Tissue-General; Methods
Biosystematic Codes:
86375 Muridae
Super Taxa:
Animals; Chordates; Vertebrates; Nonhuman Vertebrates; Mammals; Nonhuman Mammals; Rodents

5/9/8 (Item 8 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

8117767 BIOSIS Number: 91038767
AUTOMATED DNA DIAGNOSTICS USING AN ELISA-BASED **OLIGONUCLEOTIDE LIGATION** ASSAY
NICKERSON D A; KAISER R; LAPPIN S; STEWART J; HOOD L; LANDEGREN U
DIV. OF BIOL., 147-75, CALIFORNIA INST. OF TECHNOL., PASADENA, CALIF. 91125.
PROC NATL ACAD SCI U S A 87 (22). 1990. 8923-8927. CODEN: PNASA
Full Journal Title: Proceedings of the National Academy of Sciences of the United States of America
Language: ENGLISH
DNA diagnostics, the detection of specific DNA sequences, will play an increasingly important role in medicine as the molecular basis of human

disease is defined. Here, we demonstrate an automated, nonisotopic strategy for DNA diagnostics using amplification of target DNA segments by the polymerase chain reaction (PCR) and the discrimination of allelic sequence variants by a colorimetric **oligonucleotide ligation** assay (OLA). We have applied the automated PCR/OLA procedure to diagnosis of common genetic diseases, such as sickle cell anemia and cystic fibrosis (.DELTA.F508 mutation), and to genetic linkage mapping of gene segments in the human T-cell receptor .beta.-chain locus. The automated PCR/OLA strategy provides a rapid system for diagnosis of genetic, malignant, and infectious diseases as well as a powerful approach to genetic linkage mapping of chromosomes and forensic DNA typing.

Descriptors/Keywords: HUMAN POLYMERASE CHAIN REACTION-COLORIMETRIC **OLIGONUCLEOTIDE LIGATION** ASSAY DNA AMPLIFICATION GENE DETECTION GENOME MAPPING MOLECULAR SEQUENCE DATA NUCLEOTIDE SEQUENCE DNA SEQUENCE SEQUENCING

Concept Codes:

*01004	Methods, Materials and Apparatus, General-Laboratory Methods
*02508	Cytology and Cytochemistry-Human
*03508	Genetics and Cytogenetics-Human
*10006	Clinical Biochemistry; General Methods and Applications
*10052	Biochemical Methods-Nucleic Acids, Purines and Pyrimidines
*10062	Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
*10300	Replication, Transcription, Translation
*10504	Biophysics-General Biophysical Techniques
*10506	Biophysics-Molecular Properties and Macromolecules
*10804	Enzymes-Methods
*12504	Pathology, General and Miscellaneous-Diagnostic
*13014	Metabolism-Nucleic Acids, Purines and Pyrimidines
*34502	Immunology and Immunochemistry-General; Methods
00530	General Biology-Information, Documentation, Retrieval and Computer Applications
01006	Methods, Materials and Apparatus, General-Laboratory Apparatus
04500	Mathematical Biology and Statistical Methods
10064	Biochemical Studies-Proteins, Peptides and Amino Acids
10511	Biophysics-Bioengineering
32600	In Vitro Studies, Cellular and Subcellular

Biosystematic Codes:

86215	Hominidae

Super Taxa:

Animals; Chordates; Vertebrates; Mammals; Primates; Humans

5/9/9 (Item 1 from file: 144)

DIALOG(R)File 144:Pascal

10360281 PASCAL No.: 92-0563741

Automatable screening of yeast artificial-chromosome libraries based on the **oligonucleotide-ligation** assay

PUI-YAN KWOK; GREMAUD M F; **NICKERSON D A**; HOOD L; OLSON M V
Washington univ. school medicine, dep. genetics, St. Louis MO 63110, USA

Journal: Genomics : (San Diego, CA), 1992, 13 (4) 935-941
ISSN: 0888-7543 Availability: INIST-21389; 354000020483050040
No. of Refs.: 11 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: USA
Language: English

English Descriptors: Chromosome DNA; Molecular cloning; Screening; Method;

Language: ENGLISH

7/7/10 (Item 10 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

6624873 BIOSIS Number: 86091424
A %LIGASE%-MEDIATED GENE DETECTION TECHNIQUE
%LANDEGREN U%; KAISER R; SANDERS J; HOOD L
DIV. BIOL., CALIF. INST. TECHNOL., PASADENA, CA 91125.
SCIENCE (WASHINGTON D C) 241 (4869). 1988. 1077-1080. CODEN: SCIEA
Full Journal Title: SCIENCE (Washington D C)
Language: ENGLISH
An assay for the presence of given DNA sequences has been developed, based on the ability of two oligonucleotides to anneal immediately adjacent to each other on a complementary target DNA molecule. The two oligonucleotides are then joined covalently by the action of a DNA %ligase% , provided that the nucleotides at the junction are correctly base-paired. Thus single nucleotide substitutions can be distinguished. This strategy permits the rapid and standardized identification of single-copy gene sequences in genomic DNA.

7/7/11 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02470678 Genuine Article#: LD552 Number of References: 49
Title: MOLECULAR MECHANICS OF NUCLEIC-ACID SEQUENCE AMPLIFICATION
Author(s): %LANDEGREN U%
Corporate Source: UNIV UPPSALA,CTR BIOMED,DEPT MED GENET/S-75123 UPPSALA//SWEDEN/
Journal: TRENDS IN GENETICS, 1993, V9, N6 (JUN), P199-204
ISSN: 0168-9525
Language: ENGLISH Document Type: ARTICLE
Abstract: Protocols for in vitro amplification of nucleic acids are proliferating and there are now several methods that will contribute both to genetic research and to the diagnosis of a wide range of diseases. Here, I present the working principles of some of these molecular machines for amplifying DNA or RNA and discuss the lines along which new methods of amplifying nucleic acids may be developed.

7/7/12 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06935715 93113322
Capture PCR: efficient amplification of DNA fragments adjacent to a known sequence in human and YAC DNA.
Lagerstrom M; Parik J; Malmgren H; Stewart J; Pettersson U; %Landegren U%
Department of Medical Genetics, Uppsala University, Sweden.
PCR Methods Appl (UNITED STATES) Nov 1991, 1 (2) p111-9, ISSN 1054-9803 Journal Code: BNV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We have devised a procedure, termed capture PCR (CPCR), that permits the rapid isolation of DNA segments situated adjacent to a characterized

Immunoenzymatic techniques applied to the specific detection of nucleic acids. A review.

Guesdon JL
Laboratorie des Sondes Froides, Institut Pasteur, Paris, France.

J Immunol Methods (NETHERLANDS) Jun 24 **1992** , 150 (1-2) p33-49, ISSN 0022-1759 Journal Code: IFE
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, ACADEMIC
JOURNAL ANNOUNCEMENT: 9210
Subfile: INDEX MEDICUS

Numerous enzymatic and chemical methods are now available for the preparation of non-radioactive **nucleic** acid probes. Labels, such as enzymes, fluorophores, lumiphores can be attached to the **nucleic** acid probe either by covalent bonds (direct labelling) or by biospecific recognition after hybridization (indirect labelling). The principle of the latter method is based on the use of a hapten-labelled **nucleic** acid probe which is generally detected by an immunoenzymatic assay. Indirect labelling has several advantages: this procedure uses multienzyme complexes to increase the number of enzyme molecules associated with hybridization and hence provides an increase in detectability; moreover, haptens (biotin, dinitrophenol, acetylaminofluorene analogues, digoxigenin, brominated or sulphonylated pyrimidines) used to label **nucleic** acid probes are not sensitive to elevated temperatures (42-80 degrees C), extended incubation times (several hours), detergents and organic solvents currently required in hybridization techniques. The application of the immunoenzymatic and related techniques to **nucleic** acid probing is reviewed, focussing on the strategies of non-radioactive hybridization, hapten-labelling of **nucleic** acids and methods for the immunodetection of the hybrids. (133 Refs.)

Tags: Support, Non-U.S. Gov't
Descriptors: Immunoenzyme Techniques; ***Nucleic** Acid Hybridization; ***Nucleic** Acids--Analysis--AN; Affinity Labels; **Nucleic** Acid Probes
CAS Registry No.: 0 (Affinity Labels); 0 (Nucleic Acid Probes); 0 (Nucleic Acids)

Detection of point mutation in bla T genes of Enterobacteriaceae by biotinylated oligonucleotide probes using microwell hybridization and enzymofluorometric method.

Tham TN; Guesdon JL
Laboratoire des Sondes Froides, Institut Pasteur, Paris, France.

Mol Cell Probes (ENGLAND) Feb 1992 , 6 (1) p79-85, ISSN 0890-8508
Journal Code: MG9
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9206
Subfile: INDEX MEDICUS

Point mutation in the nucleotide sequence of the structural genes for the TEM-type penicillinases can broaden their substrate spectrum towards all beta-lactams except cephamicins and imipenem. We describe here hybridization techniques for the detection of point mutations by non-radioactive oligonucleotide probes with plasmid DNA carrying bla T genes immobilized in polystyrene microwells. After hybridization in discriminating conditions with corresponding biotinylated oligonucleotide probes, the hybrids were detected by using a streptavidin-alkaline phosphatase conjugate and a fluorogenic substrate, 4-methylumbelliferyl-phosphate. The adsorption of DNA to microwells used in the present work was found to be independent of Mg2+ and Na+ concentrations. By this method, less than 5 fmols of target DNA were sufficient for the detection of point mutation.

Descriptors: Enterobacteriaceae--Genetics--GE; *Fluorometry--Methods--MT; *Genes, Bacterial--Genetics--GE; *Mutation--Genetics--GE; *Nucleic Acid Hybridization; Adsorption; Base Sequence; DNA, Bacterial--Chemistry--CH; Molecular Sequence Data; Oligonucleotide Probes
CAS Registry No.: 0 (DNA, Bacterial); 0 (Oligonucleotide Probes)
Gene Symbol: bla T-1; bla T-3

DNA enzyme immunoassay: **a rapid and convenient colorimetric method for diagnosis** of cystic **fibrosis**.

Mazza C; Mantero G ; Primi D
Consiglio Nazionale delle Ricerche (CNR), Medical School, University of Brescia, Italy.

Mol Cell Probes (ENGLAND) Dec **1991** , 5 (6) p459-66, ISSN 0890-8508
Journal Code: MCP
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9205
Subfile: INDEX MEDICUS

Genetic analysis of inherited diseases has been greatly facilitated by new approaches, involving genomic DNA amplification by the polymerase chain reaction (PCR), followed by hybridization with wild type-specific or mutation-specific oligonucleotide (MSO) probes. The main advantage of these methods is that they allow easy detection of point mutations starting from minimal amounts of biological materials. These techniques, however, require procedures which are not well suited to large-scale screening or use in routine laboratories. The development of dedicated kits to perform these tests efficiently in clinical laboratories is an important current issue. We developed a new non-radioisotopic assay to reveal specifically DNA-DNA hybrids between amplified DNA and MSO probes, and applied it to the detection of two mutations causing cystic fibrosis. The detection of hybrids is achieved by means of an anti double-stranded DNA antibody, in a format which is designed as a colorimetric assay resembling a common enzymatic immunoassay (EIA). The assay detects the hybridization event, independent of the nucleic acid sequences involved in the formation of the specific hybrids, and can be used with any combination of target DNA and probes. Therefore, this test represents a significant improvement for the clinical use of the polymerase chain reaction in the diagnosis of inherited diseases.

Tags: Human; Support, Non-U.S. Gov't

DNA enzyme immunoassay : general method for detecting products of polymerase chain reaction.

Mantero G ; Zonaro A; Albertini A; Bertolo P; Primi D

Consorzio per le Biotecnologie, Consiglio Nazionale delle Ricerche (CNR), Institute of Chemistry, Medical School, Brescia, Italy.

Clin Chem (UNITED STATES) Mar **1991** , 37 (3) p422-9, ISSN 0009-9147

Journal Code: DBZ

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9106

Subfile: INDEX MEDICUS

We developed a new colorimetric method, DNA enzyme **immunoassay** (DEIA), for detecting specific hybrids of complementary nucleic acids and applied it to the detection of hepatitis B virus (HBV) DNA amplified from serum samples by means of the polymerase chain reaction (PCR) technique. The method is based on the ability of an anti-DNA monoclonal antibody to discriminate between single-stranded and double-stranded DNA. A solid phase was coated with a specific oligonucleotide probe, internal to the amplified region of HBV DNA, via an avidin-biotin bridge. The denatured PCR product was hybridized with the solid-phase probe, and the amplified DNA probe hybrid was then incubated with a monoclonal antibody specific for double- but not single-stranded DNA. Colorimetric detection of the DNA-antibody complex was achieved by adding an anti-mouse Ig antibody labeled with horseradish peroxidase. The combined use of DEIA and PCR can reveal a few HBV genome copies present in a serum sample. This method has several advantages: (a) the sensitivity is adequate for the detection of amplified DNA; (b) the signal is associated with the hybridization event, independently of modifications of the probe or of the amplification primers; and (c) the test is simple and rapid and, most importantly, requires only the standard facilities of a routine clinical laboratory.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: *DNA--Blood--BL; *DNA Probes; *Hepatitis B Virus--Genetics --GE; *Polymerase Chain Reaction; Antibodies, Anti-Idiotypic--Isolation and Purification--IP; Base Sequence; Blotting, Southern; Colorimetry--Methods --MT; Immunoenzyme Techniques; Molecular Sequence Data

CAS Registry No.: 0 (Antibodies, Anti-Idiotypic); 0 (DNA Probes); 9007-49-2 DNA

Automated DNA diagnostics using an ELISA-based oligonucleotide ligation assay.

Nickerson DA ; Kaiser R; Lappin S; Stewart J; Hood L; Landegren U
Division of Biology, California Institute of Technology, Pasadena 91125.

Proc Natl Acad Sci U S A (UNITED STATES) Nov **1990** , 87 (22) p8923-7, ISSN 0027-8424 Journal Code: PV3
Contract/Grant No.: HG 00084, HG, NHGRI
Languages: ENGLISH
Document Type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9103
Subfile: INDEX MEDICUS

DNA diagnostics, the detection of specific DNA sequences, will play an increasingly important role in medicine as the molecular basis of human disease is defined. Here, we demonstrate an automated, nonisotopic strategy for DNA diagnostics using amplification of target DNA segments by the polymerase chain reaction (PCR) and the discrimination of allelic sequence variants by a colorimetric oligonucleotide **ligation** assay (OLA). We have applied the automated PCR/OLA procedure to diagnosis of common genetic diseases, such as sickle cell anemia and cystic fibrosis (delta F508 mutation), and to genetic linkage mapping of gene segments in the human T-cell receptor beta-chain locus. The automated PCR/OLA strategy provides a rapid system for diagnosis of genetic, malignant, and infectious diseases as well as a powerful approach to genetic linkage mapping of chromosomes and forensic DNA typing.

Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.
Descriptors: *DNA--Genetics--GE; *Oligonucleotides--Diagnostic Use--DU; *Polymerase Chain Reaction--Methods--MT; Base Sequence; Enzyme-Linked Immunosorbent Assay--Methods--MT; Haplotypes; **Ligation** ; Linkage (Genetics); Molecular Sequence Data; Polymorphism (Genetics); Robotics
CAS Registry No.: 0 (Oligonucleotides); 9007-49-2 (DNA)

Detection of hepatitis B virus DNA in serum by polymerase chain reaction amplification and microtiter sandwich hybridization.

Keller GH ; Huang DP; Shih JW; Manak MM

Biotech . esearch Laboratories, Inc., Rockville, Maryland 20850.

J Clin Microbiol (UNITED STATES) Jun **1990** , 28 (6) p1411-6, ISSN 0095-1137 Journal Code: HSH

Language: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL . NNOUNCEMENT: 9011

Subfile: INDEX MEDICUS

We have developed a microtiter sandwich hybridization assay for the detection of polymerase chain reaction (PCR)-amplified **hepatitis** B virus (HBV) sequences. This assay utilizes an enzyme-linked immunosorbent assay-like format in which cloned DNA containing a sequence complementary to half of one PCR product strand is immobilized in microtiter wells. A biotin-labeled DNA sequence complementary to the other portion of the same PCR product strand is used as the probe. The DNAs from 69 **hepatitis** B surface antigen-positive serum samples and 16 antigen-negative control samples were amplified by the PCR procedure, and the product was detected by Southern and sandwich hybridization. Both detection procedures were capable of detecting as few as five copies of HBV DNA. Compared with Southern hybridization, the sandwich hybridization assay exhibited a sensitivity of 100% and a specificity of 95% for the detection of amplified HBV sequences. Unlike Southern hybridization, however, the sandwich hybridization assay employs a nonradioactive probe and allows easy handling of large numbers of samples. DNA was detected in 74% of the antigen-positive samples. All of the antigen-negative samples (healthy blood donors) were negative for HBV DNA by both procedures.

Tags: Human

NOVEL ULTRASENSITIVE COLORIMETRIC ASSAY FOR DNA

TRISCOTT MX
ELCATECH INC, 64-1001 S MARSHALL ST, WINSTON-SALEM, NC 27101
Source: Crisp Data Base National Institutes Of Health
Language: ENGLISH
Document Type: Research
Spon. Agency: U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INST. OF HEALTH, NATIONAL CANCER INSTITUTE
Contract Number: 1R43CA62468-01
Award Type: Grant
Journal Announcement: 9403
RPROJ/CRISP The detection of specific DNA sequences for the diagnosis of genetic diseases, cancer, and infectious disease is becoming increasingly widespread. A number of innovative techniques have been employed, including the oligonucleotide **ligation** assay and reverse dot-blot hybridization. The end-point, and to a certain extent the effectiveness of these assays is determined by the sensitivity of the DNA detection techniques used. Progress has been significantly enhanced through the use of the polymerase chain reaction (PCR). However, implementation of PCR can be expensive, as well as equipment and expertise intensive. We have applied a technique with proven effectiveness in the field of **immunoassays** for the non-isotopic detection of DNA; initial data indicates an increase in sensitivity 10(2)-10(3) times higher than conventional techniques. The assay, which we have called EDNA-ELCA, uses the amplification of the coagulation cascade to produce a colorimetrically detectable end product. A DNA template is labelled with biotin and a **haptene** , and is then immobilised in a streptavidin coated microtiter plate. An antibody against the **haptene** is conjugated to a snake venom coagulation activator (RVVXa), and introduced into the microtiter well. The assay is then developed by the addition of coagulation factors, including an enzyme labelled fibrinogen. The deposition of enzyme labelled fibrin is indicative of a positive reaction. In this proposal we have chosen three specific applications to prove the utility of this method. They are detection of the deltaF508 mutation of cystic fibrosis, a codon 12 mutation in the first exon of K- ras for colon cancer, and the mecA gene in methicillin resistant Staphlococcus aureus.

Descriptors/Keywords: blood coagulation; clotting factor; fibrinogen; thrombin; polymerase chain reaction; gene mutation; DNA; colorimetry; binding protein; reptile poison; biotin; method development; CRISP; RPROJ

11/9/11 (Item 11 from file: 94)

DIALOG(R)File 94:JICST-EPlus

00954487 JICST ACCESSION NUMBER: 89A0599886 FILE SEGMENT: JICST-E

I-J as a second T cell receptor for self-molecular polymorphism and the role in suppressive signal transduction.

TADA T (1); NAKAYAMA T (1); ASANO Y (1); KISHIMOTO H (1); SANO K (1)
(1) Univ. Tokyo, Tokyo, JPN
Proc Int Symp Princess Takamatsu Cancer Res Fund, 1989, VOL.19th(1988), PAGE.227-235, FIG.2, TBL.1, REF.25
JOURNAL NUMBER: X0389AAT
UNIVERSAL DECIMAL CLASSIFICATION: 591.111.1.05 L 577.1:576.8.097.2
LANGUAGE: English COUNTRY OF PUBLICATION: Japan
DOCUMENT TYPE: Conference Proceeding
ARTICLE TYPE: Review article
ABSTRACT: We have been able to demonstrate an I-J **epitope** on the cell surface of interleukin 2(IL-2) dependent T cell clones of both T helper(Th) and suppressor T cells(Ts) lineages by flow microfluorometry with fluorescinated anti-I-Jk, and to identify the molecule by the specific immunoprecipitation and the gel analysis. The I-J molecule thus detected is a novel 90K dimeric molecule composed of 45K glycopeptide subunits distinct from conventional T cell receptor(TcR) or CD28. The molecule is not comodulated with TcR or T3 on the cell surface. Monoclonal anti-I-J can inhibit various major histocompatibility complex(MHC)-restricted T cell functions including Ca2+ influx, antigen-induced T cell proliferation and IL-2 production,

indicating that the **ligation** of I-J molecules results in a negative signal transduction. Since it has been reported that the I-J phenotype undergoes a systematic adaptive alteration in radiation bone marrow chimeras in association with the acquired class II restriction, it is likely that I-J is a novel receptor for self MHC apart from TcR heterodimer and is required for a negative signal transduction.(author abst.)
DESCRIPTORS: helper T cell; suppressor T cell; interleukin 2; receptor; surface antigen; histocompatibility antigen; transduction(genetics); gel electrophoresis; immunoprecipitation; dimer; glycoprotein; **epitope**

BROADER DESCRIPTORS: T lymphocyte; lymphocyte; leucocyte; blood corpuscle; blood; body fluid; cell(cytology); interleukin; growth factor; bioactive factor; factor; lymphokine; cytokine; antigen; isoantigen; bacteriophage infection; viral infection; infection; electrophoresis; **immunoassay** ; bioassay; multimer; protein
CLASSIFICATION CODE(S): EJ03030H; ED02020X

11/9/12 (Item 12 from file: 94)
DIALOG(R)File 94:JICST-EPlus

00573294 JICST ACCESSION NUMBER: 88A0054606 FILE SEGMENT: JICST-E
Clinical immunology necessary for understanding collagen diseases. Autoantibody. With special reference to antinuclear antibody.
AKIZUKI MASASHI (1); MIMORI TSUNEYO (1); YAMAGATA HAJIME (2)
(1) Keio Univ.; (2) National Sanatorium Murayama Hospital
Medicina, 198 , VOL.24,NO.8, PAGE.1345-1351, TBL.3, REF.36
JOURNAL NUMBER: Z0074BAR ISSN NO: 0025-7699
UNIVERSAL DECIMAL CLASSIFICATION: 577.1:576.8.097.5 616-008/-009
LANGUAGE: Japanese COUNTRY OF PUBLICATION: Japan
DOCUMENT TYPE Journal
ARTICLE TYPE: Commentary
DESCRIPTORS: human(primates); collagen disease; autoantibody; antinuclear antibody; rheumatoid factor; serum concentration; isoantigen; fluorescent antibody technique; immunodiffusion; protein nucleic acid complex; DNA; histone; aminoacyl-tRNA synthetase; **epitope**
BROADER DESCRIPTORS: Primates; Mammalia; Vertebrata; animal; disease; isoantibody; antibody; factor; blood concentration; concentration(ratio); degree; antigen; labeled antibody method; **immunoassay** ; bioassay; immunoprecipitation; protein complex; complex(substance); nucleic acid; deoxyribonucleoprotein; nucleoprotein ; protein; C-O **ligase** ; **ligase** ; enzyme
CLASSIFICATION CODE(S): ED02030I; GD07010X
?logoff hold

Competitor template RNA for detection and quantitation of hepatitis A virus by PCR

Goswami B B ; Koch W H; Cebula T A
Div. Molecular Biol. Res. and Evaluation, Food Drug Adm., 200 C St. S.W., Washington, DC 20204, USA

Biotechniques 16 (1). 1994. 114-115, 118, 120-121.
Full Journal Title: Biotechniques
ISSN: 0736-6205
Language: ENGLISH
Print Number: Biological Abstracts Vol. 097 Iss. 007 Ref. 091815

PCR was used to introduce a 63-bp deletion into the putative RNA replicase coding sequence of hepatitis A virus. RNA was synthesized in vitro from the deletion mutant cloned into a transcription vector. Upon amplification by PCR, cDNA made from the competitor RNA generated an amplified fragment that could be easily distinguished from the product generated from wild-type hepatitis A virus genomic RNA by gel electrophoresis, when the same primers were used, without further manipulation. The competitor RNA was used as a positive control in PCR-based detection of very low copy numbers of hepatitis A virus genomic RNA in the presence of unrelated hard-shell clam RNA. When the competitor RNA was used for competitive PCR to quantitate wildtype RNA, the presence of one template at a 10-fold to 100-fold higher level almost completely inhibited product formation from the underrepresented template. The competitor RNA should be useful as a control for reverse transcription and PCRs to determine hepatitis A virus genome RNA when accidental contamination of test samples by a wild-type positive control template would compromise the results.

Descriptors/Keywords: RESEARCH ARTICLE; REVERSE TRANSCRIPTION; AMPLIFICATION; POLYMERASE CHAIN REACTION; ANALYTICAL METHOD
Concept Codes:
*10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines
*10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
*10300 Replication, Transcription, Translation
*10804 Enzymes-Methods

WO93/20227 1993

US PAT NO: 5,753,439 [IMAGE AVAILABLE] L6: 1 of 3

SUMMARY:

BSUM(6)

Many genetic defects such as Burkett's lymphoma and some sickle cell anemia and thalassemia mutations are detectable without the use of sequencing. Such techniques include restriction fragment length polymorphism (RFLP) and chromosome karyotyping. However, general applicability of these methods is limited as most genetic defects are more modest and do not alter restriction sites or cause chromosome rearrangements. Polymerase chain reaction and **ligase** chain reaction can increase the sensitivity of many detection methods and detect single base pair changes in nucleic acid. However, if the mutation involves repeated sequences, the degeneracy of the repeated sequence makes even PCR and LCR detections unreliable.

DETDESC:

DETD(11)

Although PCR is a reliable method for amplification of target sequences, a number of other techniques can be used such as isothermic amplification, **ligase** chain reaction (LCR), self sustained sequence replication (3SR), polymerase chain reaction linked **ligase** chain reaction (pLCR), gaped **ligase** chain reaction (gLCR), **ligase** chain detection (LCD). The principle of **ligase** chain reaction is based in part on the **ligation** of two adjacent synthetic **oligonucleotide** primers which uniquely hybridize to one strand of the target DNA or RNA. If the target is present, the two **oligonucleotides** can be covalently linked by **ligase**. A second pair of primers, almost entirely complementary to the first pair of primers is also provided in a **ligase** chain reaction. In a **ligase** chain reaction, the template and the four primers are placed into a thermocycler with thermostable **ligase**. As the temperature is raised and lowered, **oligonucleotides** are renatured adjacent to each other on the template and **ligated**. The **ligated** product of one reaction serves as the template for a subsequent round of **ligation**. The presence of target is manifested as a DNA fragment with a length equal to the sum of the two adjacent **oligonucleotides**. Additional PCR variations include in situ PCR and **immuno-PCR** amplification which utilizes nucleic acid fragments **coupled** to pathogen-specific antibodies to increase detection sensitivity. Alternatively, nucleic acids can be analyzed after purification using, for example, DNA or RNA polymerases, PCR or another amplification technique. PCR analysis of RNA, or RT-PCR, involves reverse transcription of RNA, such as mRNA sequences, into cDNA copies. These target cDNA sequences are hybridized to primers which amplify the nucleic acid using PCR amplification.

Cited Reference

DETDESC:

DETD(54)

A plurality of probes, each containing 5' and a 3' sequence complementary to the target nucleic acid and from 10 to 109 internal repeats are synthesized on an oligonucleotide synthesizer. Probes of 80 bases or shorter are synthesized and used directly. Probes greater than

u et al., Genomics 4:560 (1989);
Backman et al., EP 320,308; Wallace, EP 336,731; Orgel, WO 89/09835).

US PAT NO: 5,011,771 [IMAGE AVAILABLE] L22: 12 of 18

SUMMARY:

BSUM(9)

These previously described **immunoassays** for AFP are of two types. First, a two site **immunoassay** based on two monoclonal antibodies directed against two separate and distinct epitopes, one of these antibodies on a solid phase and the other in soluble labelled form. Second, a **three site immunoassay** based on three monoclonal antibodies directed against three separate and distinct epitopes, one of these antibodies on a solid phase and both others in soluble labelled form. None of the data has reported a **three site immunoassay** based on two different monoclonal antibodies directed against two distinct and separate epitopes on said antigen, the first of these antibodies on a solid phase, and the second on the solid phase and in s

Immuno- PCR with a commercially available avidin system [letter]

Ruzicka V ; Marz W; Russ A; Gross W

Science (UNITED STATES) Apr 30 **1993** , 260 (5108) p698-9, ISSN 0036-8075 Journal Code: UJ7

Languages: ENGLISH

Document type: LETTER

JOURNAL ANNOUNCEMENT: 9307

Subfile: INDEX MEDICUS

Descriptors: *Antigens--Analysis--AN; *Avidin; *Immunoassay--Methods--MT; *Polymerase Chain Reaction--Methods--MT; Biotin; DNA

CAS Registry No.: 0 (Antigens); 1405-69-2 (Avidin); 58-85-5 (Biotin); 9007-49-2 (DNA)

1. 5,679,509, Oct. 21, 1997, Methods and a diagnostic aid for distinguishing a subset of HPV that is associated with an increased risk of developing cervical dysplasia and cervical cancer; Cosette M. Wheeler, et al., 435/5, 6, 7.21, 7.23, 91.2; 436/64, 811, 813 [IMAGE AVAILABLE]

2. 5,601,978, Feb. 11, 1997, Oligonucleotides and methods for the detection of chlamydia trachomatis; John D. Burczak, et al., 435/6, 91.2; 536/24.32, 24.33; 935/77, 78 [IMAGE AVAILABLE]

3. 5,583,001, Dec. 10, 1996, Method for detection or quantitation of an analyte using an analyte dependent enzyme activation system; Mark N. Bobrow, et al., 435/7.5, 7.1, 7.7, 7.71, 7.72, 7.9, 7.91, 15, 18, 21, 25, 28; 436/501, 518 [IMAGE AVAILABLE]

4. 5,508,167, Apr. 16, 1996, Methods of screening for Alzheimer's disease; Allen D. Roses, et al., 435/6, 4, 91.2, 91.52 [IMAGE AVAILABLE]

5. 4,604,364, Aug. 5, 1986, Bioluminescent tracer composition and method of use in immunoassays; Kenneth M. Kosak, 436/501; 435/7.21, 7.32, 7.4, 7.9, 7.93, 7.94, 8, 188, 968; 436/50, 56, 172, 544, 546, 800 [IMAGE AVAILABLE]

=> d his

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     (FILE 'USPAT' ENTERED AT 10:37:43 ON 27 AUG 1998)
L1          1096 S (LIGASE? OR LIGAT?)/CLM
L2          1427 S ?IMMUNOASSAY?/CLM
L3        382758 S (THIRD? OR THREE?)/CLM
L4        384982 S L1 OR L2 OR L3
L5             0 S L1 AND L2 AND L3
```

1. 5,753,439, May 19, 1998, Nucleic acid detection methods; Cassandra L. Smith, et al., 435/6, 5, 91.2; 536/24.3, 24.32, 24.33 [IMAGE AVAILABLE]

2. 5,635,602, Jun. 3, 1997, Design and synthesis of bispecific DNA-antibody conjugates; Charles R. Cantor, et al., 530/391.1, 387.3, 391.5, 391.9; 536/23.1 [IMAGE AVAILABLE]

3. 5,384,255, Jan. 24, 1995, Ubiquitin carrier enzyme E2-F1, purification, production, and use; Aaron J. Ciechanover, et al., 435/193, 7.4, 172.1, 172.3, 252.3, 320.1; 536/23.2 [IMAGE AVAILABLE]

```
Set     Items   Description
S1         68   AU=LANDEGREN ? AND (LIGASE? OR LIGAT?)
S2         38   RD (unique items)
S3         21   S2/1996:1998
S4         17   S2 NOT S3
S5         17   TARGET - S4
S6          1   S5 AND (MONOCLONAL? OR ANTIBOD? OR IMMUNOASSAY?)
? t s6/3/1


 6/3/1     (Item 1 from file: 654)
DIALOG(R)File 654:US Pat.Full.
(c) format only 1998 The Dialog Corp. All rts. reserv.

             01941497
Utility
METHOD OF DETECTING A NUCLEOTIDE CHANGE IN NUCLEIC ACIDS
[DNA OR RNA SEQUENCE DETERMINATION FOR POSSIBLE MUTANTS, ANNEALING A TARGET
NUCLEOTIDE, CROSSLINKING, DETECTION]

PATENT NO.:  4,988,617
ISSUED:      January 29, 1991 (19910129)
INVENTOR(s): Landegren, Ulf, Pasadena, CA (California), US (United States
             of America)
             Hood, Leroy, Pasadena, CA (California), US (United States of
             America)
ASSIGNEE(s): California Institute of Technology, (A  U.S. Company or
             Corporation ), Pasadena, CA (California), US (United States of
             America)
             [Assignee Code(s): 13190]
APPL. NO.:   7-173,280
FILED:       March 25, 1988 (19880325)
FULL TEXT:         1063 lines
? s s5 not s6

              17  S5
               1  S6
      S7      16  S5 NOT S6
? t s7/7/1-16


 7/7/1     (Item 1 from file: 5)
DIALOG(R)File   5:BIOSIS PREVIEWS(R)
(c) 1998 BIOSIS. All rts. reserv.

11344938     BIOSIS Number: 97544938
  Padlock Probes: Circularizing Oligonucleotides for Localized DNA
Detection
  Nilsson M; Malmgren H; Samiotaki M; Kwiatkowski M; Chowdhary B P;
%Landegren U%
  Beijer Lab., Dep. Med. Genet., Box 589, Biomed. Cent., S-75123 Uppsala,
SWE
  Science (Washington D C) 265 (5181). 1994.  2085-2088.
  Full Journal Title:  Science (Washington D C)
  ISSN: 0036-8075
  Language: ENGLISH
  Print Number: Biological Abstracts Vol. 098 Iss. 012 Ref. 162561
  Nucleotide  sequence  information  derived from DNA segments of the human
```

reactions between oligonucleotide probes; (2) Two allele-specific probes, differentially labeled with either of two lanthanide labels, compete for %ligation% to a third oligonucleotide (the signal from the two labeled probes can thus be directly compared in a sensitive time-resolved fluorescence detection reaction); and (3) Large sets of analyses are processed in parallel using a 96-pin capture manifold, serving to reduce pipetting steps and the risk of contamination. We present here the basis of the technique and its application to the screening for two common mutations causing cystic fibrosis and alpha-1-antitrypsin deficiency.

7/7/4 (Item 4 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

10898649 BIOSIS Number: 97098649
%Ligation%-based DNA diagnostics
%Landegren U%
Dep. Med. Genetics, Box 589 BMC, Univ. Uppsala, S-75123 Uppsala, SWE
Bioessays 15 (11). 1993. 761-765.
Full Journal Title: Bioessays
ISSN: 0265-9247
Language: ENGLISH
Print Number: Biological Abstracts/RRM Vol. 046 Iss. 003 Ref. 026801

7/7/5 (Item 5 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

9545646 BIOSIS Number: 94050646
DETECTION OF MUTATIONS IN HUMAN DNA
%LANDEGREN U%
DEP. MED. GENET., BOX 589, UPPSALA BIOMED. CENT., S-75123 UPPSALA, SWED.
GENET ANAL TECH APPL 9 (1). 1992. 3-8. CODEN: GATAE
Language: ENGLISH
Efficient methods for the detection of mutations are of fundamental importance in research and in diagnostics. By detection of a DNA sequence alteration that cosegregates with a clinical phenotype in an affected family, the gene at fault may be identified and assigned a function. Mutation detection methods are also a rate-limiting factor for the clinical application of DNA diagnostics. Currently a large number of techniques are in use to scan for new mutations and to distinguish among previously established sequence variants. Here, some of the problems connected with mutation detection are discussed together with principles on which current and future mutation detection assays can be based.

7/7/6 (Item 6 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

8828068 BIOSIS Number: 42053068
A SYSTEM FOR EFFICIENT GENETIC ANALYSES USING PCR FOLLOWED BY %LIGASE% -MEDIATED GENE DETECTION AND VISUALIZATION BY TIME-RESOLVED FLUORESCENCE
SAMIOTAKIS M; PARIK J; KWIATKOWSKI M; LAGERQUIST A; PETTERSSON U; %LANDEGREN U%
DEP. MED. GENET., UPPSALA UNIV. SWED., UPPSALA, SWED.
PROCEEDINGS OF THE 8TH INTERNATIONAL CONGRESS OF HUMAN GENETICS,

WASHINGTON, D.C., USA, OCTOBER 6-11, 1991. AM J HUM GENET 49 (4 SUPPL.). 1991. 194. CODEN: AJHGA
Language: ENGLISH

7/7/7 (Item 7 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

8320405 BIOSIS Number: 41004405
OLIGONUCLEOTIDE %LIGATION% ASSAY
%LANDEGREN U%; KAISER R; HOOD L
DEP. MED. GENETICS, BIOMED. CENT., S751 NO. 23 UPPSALA, SWEDEN.
INNIS, M. A., ET AL. (ED.). PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS. XVIII+482P. ACADEMIC PRESS, INC.: SAN DIEGO, CALIFORNIA, USA; LONDON, ENGLAND, UK. ILLUS. ISBN 0-12-372181-4(PAPER); ISBN 0-12-372180-6(CLOTH). 0 (0). 1990. 92-98. CODEN: 33040
Language: ENGLISH

7/7/8 (Item 8 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

8117767 BIOSIS Number: 91038767
AUTOMATED DNA DIAGNOSTICS USING AN ELISA-BASED OLIGONUCLEOTIDE %LIGATION% ASSAY
NICKERSON D A; KAISER R; LAPPIN S; STEWART J; HOOD L; %LANDEGREN U%
DIV. OF BIOL., 147-75, CALIFORNIA INST. OF TECHNOL., PASADENA, CALIF. 91125.
PROC NATL ACAD SCI U S A 87 (22). 1990. 8923-8927. CODEN: PNASA
Full Journal Title: Proceedings of the National Academy of Sciences of the United States of America
Language: ENGLISH
DNA diagnostics, the detection of specific DNA sequences, will play an increasingly important role in medicine as the molecular basis of human disease is defined. Here, we demonstrate an automated, nonisotopic strategy for DNA diagnostics using amplification of target DNA segments by the polymerase chain reaction (PCR) and the discrimination of allelic sequence variants by a colorimetric oligonucleotide %ligation% assay (OLA). We have applied the automated PCR/OLA procedure to diagnosis of common genetic diseases, such as sickle cell anemia and cystic fibrosis (.DELTA.F508 mutation), and to genetic linkage mapping of gene segments in the human T-cell receptor .beta.-chain locus. The automated PCR/OLA strategy provides a rapid system for diagnosis of genetic, malignant, and infectious diseases as well as a powerful approach to genetic linkage mapping of chromosomes and forensic DNA typing.

7/7/9 (Item 9 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

7293280 BIOSIS Number: 38073801
OLIGONUCLEOTIDE %LIGATION% TECHNIQUE
%LANDEGREN U%
CALIFORNIA INST. TECHNOLOGY, PASADENA, CALIF. 91125.
BIOMED PHARMACOTHER 43 (9). 1989. 703-704. CODEN: BIPHE
Full Journal Title: Biomedicine & Pharmacotherapy

nucleotide sequence. In this procedure, a DNA sample is restriction-digested and a linker, comprising two base-paired oligonucleotides, is added to the ends by %ligation%. Multiple extension reactions are performed using a biotinylated primer derived from the known sequence, permitting the subsequent isolation of extension products on a streptavidin-coated support. The enriched fragments are amplified exponentially using another specific oligonucleotide, hybridizing 3' to the biotinylated primer in combination with one of the linker oligonucleotides, now functioning as a PCR primer. The convenience of CPCR is greatly enhanced by using a novel streptavidin-coated manifold, which is constructed so that it projects into each individual well of a microtiter plate. The procedure permits the simultaneous isolation of fragments from large numbers of DNA samples and minimizes the risk of contamination between reactions. We have applied this method to identify DNA sequences located downstream of known sequences in the human genome. The technique has also been used to identify end fragments of sequences cloned in a yeast artificial chromosome (YAC) vector. The reactions can be initiated directly from yeast colonies and provide access to DNA sequence information for these end fragments in a minimal number of steps. With the aid of the present technique, we have isolated over 100 end fragments from YACs derived from the human X chromosome. Isolated end sequences have been used to order YAC clones into a contig.

7/7/13 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

9179839 EMBASE No: 94118141
Detection of point mutations by solid-phase methods
Syvanen A.-C.; %Landegren U.%
Dept. of Human Molecular Genetics, National Public Health Institute, Mannerheimintie 166, SF-00300 Helsinki Finland
HUM. MUTAT. (USA) , 1994, 3/3 (172-179)
CODEN: HUMUE ISSN: 1059-7794
LANGUAGES: English SUMMARY LANGUAGES: English
Several techniques exist that permit the efficient distinction among characterized DNA sequence variants. In this review we discuss a number of such analytic procedures. These techniques all take advantage of a variety solid supports to prepare and analyze reaction products. The described diagnostic principles are now being applied for the development of miniaturized assay formats, suitable for automated detection of large sets of sequences in clinical samples.

7/7/14 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

8879757 EMBASE No: 93183489
1.5-Mb YAC contig in Xq28 formatted with sequence-tagged sites and including a region unstable in the clones
Palmieri G.; Romano G.; Casamassimi A.; D'Urso M.; Little R.D.; Abidi F.E.; Schlessinger D.; Lagerstrom M.; Malmgren H.; Steen-Bondeson M.- L.; Pettersson U.; %Landegren U.%
Intl. Genetics/Biophysics Institute, Via Marconi, 10, Naples Italy
GENOMICS (USA) , 1993, 16/3 (586-592)
CODEN: GNMCE ISSN: 0888-7543
LANGUAGES: English SUMMARY LANGUAGES: English

A contig of 20 yeast artificial clones (YACs) has been assembled across 1.5 Mb of Xq28 and formatted with nine previously reported probes and nine STSs developed from the sequence of probes and end fragments of YACs. YAC end fragments were obtained by subcloning, Alu-vector PCR, or primer-%ligation% PCR methods. Eighteen of the YACs were recovered from a library specific for Xq24-q28; two that fill a gap were obtained from a second library made from total human DNA. One region, containing probes pX78c and 2A1.1, was unstable in YACs, but it was possible to generate a self-consistent map of DNA over the entire contig. Overlaps were confirmed by Southern blot analyses of YAC DNAs, and pulsed-field gel electrophoresis confirmed the extent of the contig and identified at least four CpG islands in the region.

7/7/15 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

2115355 9102646
C/ METHOD OF DETECTING A NUCLEOTIDE CHANGE IN NUCLEIC ACIDS; DNA OR RNA SEQUENCE DETERMINATION FOR POSSIBLE MUTANTS, ANNEALING A TARGET NUCLEOTIDE, CROSSLINKING, DETECTION
Document Type: UTILITY
Inventors: Hood Leroy (US); %Landegren Ulf% (US
Assignee: California Institute of Technology Assignee Code: 13190

	Patent Number	Issue Date	Applic Number	Applic Date
Patent:	US 4988617	910129	US 173280	880325
	(Cited in 006 later patents)			
Priority Applic:			US 173280	880325

Abstract:
Assay for determing the nucleic acid sequence in a region of a nucleic acid test substance having a known normal sequence and a known possible mutation at at least one target nucleotide position. Oligonucleotide probes are selected to anneal to immediately adjacent segments of a substantially complementary test DNA or RNA molecule. The target probe has an end region wherein one of the end region nucleotides is complementary to the normal or abnormal nucleotide at the corresponding target nucleotide position. A linking agent is added under conditions such that when the target nucleotide is correctly base paired, the probes are covalently joined and if not correctly base paired, the probes are incapable of being covalently joined under such conditions. The presence or absence of linking is detected as an indication of the sequence of the target nucleotide.

Exemplary Claim:
1. An assay for a biologically derived denatured DNA or RNA test substance, which has a known normal nucleotide sequence and a known possible mutation at at least one target nucleotide position in said sequence, which assay determines whether the test substance has said normal nucleotide sequence or said possible mutation, said assay comprising the steps of (a) annealing a target oligonucleotide probe of predetermined sequence to a first sequence of said test substance so that said target nucleotide position is aligned with a nucleotide in an end region of said target probe, (b) annealing an adjacent oligonucleotide probe of predetermined sequence to a second sequence of said test substance contiguous to said first sequence, so that the terminal nucleotide in said end region of said target probe and one end of said adjacent probe are directly adjacent to each other, (c) contacting said annealed target

probe and adjacent probe with a linking agent under conditions such that the directly adjacent ends of said probes covalently bond to form a linked probe product unless there is nucleotide base pair mismatching between said target probe and said test substance at the target nucleotide position, (d) separating said test substance and linked probe product, if formed, and (e) detecting whether or not said linked probe product is formed as an indication of nucleotide base pair matching or mismatching at said target nucleotide position.

7/7/16 (Item 1 from file: 348)
DIALOG(R)File 348:European Patents

00642399
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
RNA ASSAYS USING RNA BINARY PROBES AND RIBOZYME %LIGASE%.
RNA-ASSAYS MITTELS BINAREN RNA PROBEN UND RIBOZYM-%LIGASE%.
DOSAGES D'ARN AU MOYEN DE SONDES BINAIRES D'ARN ET D'UNE RIBOZYME-%LIGASE%.

PATENT ASSIGNEE:
THE PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC., (378160), 455 First Avenue, New York NY 10016, (US), (applicant designated states: DE;FR;GB;IT)
THE GENERAL HOSPITAL CORPORATION, (370409), Fruit Street, Boston, Massachusetts 02114, (US), (applicant designated states: DE;FR;GB;IT)
INVENTOR:
LIZARDI, Paul M., Privada Cerritos 99, Colonia Rancho Cortez, Cuernavaca 62120, (MX)
TYAGI, Sanjay, Apartment 4N, 485 First Avenue, New York, NY 10016, (US)
%LANDEGREN, Ulf D.%, Eksoppsvagen 16, S-75646 Uppsala, (SE)
KRAMER, Fred R., 561 West 231 Street, Riverdale, NY 10463, (US)
SZOSTAK, Jack W., Apartment 6L, 308 Commonwealth Avenue, Boston, MA 02115 , (US
LEGAL REPRESENTATIVE:
Bizley, Richard Edward et al (28352), Hepworth, Lawrence, Bryer & Bizley Merlin House Falconry Court Bakers Lane, Epping Essex CM16 5DQ, (GB)
PATENT (CC, No, Kind, Date): EP 682716 A1 951122 (Basic)
WO 9416105 940721
APPLICATION (CC, No, Date): EP 94907210 940114; WO 94US470 940114
PRIORITY (CC, No, Date): US 5893 930115
DESIGNATED STATES: DE; FR; GB; IT
INTERNATIONAL PATENT CLASS: C12Q-001/68; C12P-019/34;
LEGAL STATUS (Type, Pub Date, Kind, Text):
Application: 941026 A International application (Art. 158(1))
Application: 951122 A1 Published application (A1with Search Report ;A2without Search Report)
Examination: 951122 A1 Date of filing of request for examination: 950815
LANGUAGE (Publication,Procedural,Application): English; English; English

se **chain reaction** which is
generally described by Weiss, Science, 254, pages 1292-3, 1991 to increase the amount of targeted nucleic acid which can then be detected using the composition of this invention.

9598783 BIOSIS Number: 94103783

AUTOMATABLE SCREENING OF YEAST ARTIFICIAL-CHROMOSOME LIBRARIES BASED ON THE **OLIGONUCLEOTIDE-LIGATION** ASSAY

KWOK P-Y; GREMAUD M F; **NICKERSON D A**; HOOD L; OLSON M V

DEP. GENETICS, WASHINGTON UNIVERSITY SCHOOL MEDICINE, ST. LOUIS, MO. 63110.

GENOMICS 13 (4). 1992. 935-941. CODEN: GNMCE

Full Journal Title: Genomics

Language: ENGLISH

The systematic screening of yeast artificial-chromosome (YAC) libraries is the limiting step in many physical mapping projects. To improve the screening throughput for a human YAC library, we designed an automatable strategy to identify YAC clones containing a specific segment of DNA. Our approach combines amplification of the target sequence from pooled YAC DNA by the polymerase chain reaction (PCR) with detection of the sequence by an ELISA-based **oligonucleotide-ligation** assay (OLA). The PCR-OLA approach eliminates the use of radioactive isotopes and gel electrophoresis, two of the major obstacles to automated YAC screening. Furthermore, the use of the OLA to test for the presence of sequences internal to PCR primers provides an additional level of sensitivity and specificity in comparison to methods that rely solely on the PCR.

Descriptors/Keywords: HUMAN POLYMERASE CHAIN REACTION METHOD NUCLEOTIDE SEQUENCE MOLECULAR SEQUENCE DATA

Concept Codes:

*03504 Genetics and Cytogenetics-Plant

*03508 Genetics and Cytogenetics-Human

*10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines

*10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines

Biosystematic Codes:

15000 Fungi-Unspecified

86215 Hominidae

Super Taxa:

Microorganisms; Plants; Nonvascular Plants; Fungi; Animals; Chordates; Vertebrates; Mammals; Primates; Humans

(FILE 'USPAT' ENTERED AT 08:05:23 ON 27 AUG 1998)
DELETE HISTORY Y

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L1          13 SEARCH (5561049 OR 5254458 OR 5026653 OR 4748111 OR 446978
7 O
L2          13 SEARCH (5459078 OR 5670381 OR 5424219 OR 4514508 OR 469089
0 O
L3          13 SEARCH (4376110 OR 5364760 OR 5656731 OR 5391272 OR 554105
9 O
L4          11 SEARCH (4486530 OR 4468470 OR 5580730 OR 5650284 OR 450314
3 O
L5          50 SEARCH L1 OR L2 OR L3 OR L4
L6           6 S L5 AND (LIGAT? OR LIGASE?)

=> d cit 1-6
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1. **5,656,731**, Aug. 12, 1997, Nucleic acid-amplified immunoassay probes; Michael S. Urdea, 530/391.1; 435/6, 7.1, 7.7, 7.72, 91.1, 810; 436/501; 530/387.1, 391.3, 391.5; 536/23.1, 24.1, 24.31, 24.32, 24.33; 935/77, 78 [IMAGE AVAILABLE]

2. **5,593,825**, Jan. 14, 1997, Hepatitis B virus mutants, reagents and methods for detection; William F. Carman, et al., 435/5, 69.3, 252.3, 254.11, 320.1, 325, 352, 362, 367, 975; 536/23.72, 24.32 [IMAGE AVAILABLE]

3. **5,364,760**, Nov. 15, 1994, Replicative RNA reporter systems; Barbara Chu, et al., 435/6, 7.1, 91.2, 188, 810; 436/501, 547, 827; 536/23.1; 935/78 [IMAGE AVAILABLE]

4. **5,254,458**, Oct. 19, 1993, Immunoassays using antigens produced in heterologous organisms; Larry T. Mimms, 435/5, 7.1, 7.92, 7.94, 973, 974; 436/820 [IMAGE AVAILABLE]

5. **4,824,775**, Apr. 25, 1989, Cells labeled with multiple Fluorophores bound to a nucleic acid carrier; Nanibhushan Dattagupta, et al., 435/4, 6, 29, 243, 355; 436/519, 546; 514/2, 44 [IMAGE AVAILABLE]

6. **4,748,111**, May 31, 1988, Nucleic acid-protein conjugate used in immunoassay; Nanibhushan Dattagupta, et al., 435/6, 7.23, 7.24, 7.95, 964; 436/518, 547, 828; 530/402 [IMAGE AVAILABLE]

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     (FILE 'USPAT' ENTERED AT 13:04:02 ON 27 AUG 1998)
L1        13235 S OLIGO# OR OLIGO(W)NUCLEOT? OR OLIGO-NUCLEOT? OR OLIGONUC
LEO
L2           18 S IMMUNOPCR OR IMMUNO-PCR OR IMMUNOASSAY? (2A) PCR
L3       966140 S CROSSLINK? OR CROSS (W) LINK? OR CROSS-LINK? OR COMPLEX?
 OR
L4            7 S L2 (P) L3
L5            3 S L1 (P) L4
L6            3 S L5 AND (LIGASE? OR LIGAT?)
L7            1 S L1 (P) L2 (P) (LIGAT? OR LIGASE?)
L8           18 S POLYMERASE(P) CHAIN(P) REACTION(P) (LIGASE OR LIGAT?) (P
) (
L9           17 S L8 NOT L2
L10          35 S L2 OR L8
L11          22 S L10 AND (EPITOPES OR DETERMINANT?)
L12           3 S L11 AND ((EPITOPES OR DETERMINANT?) (P) (THREE OR THIRD)
)
L13           2 S (((THREE OR THIRD) (5A)EPITOP?) OR ((THREE OR THIRD) (5A
) D
L14          12 S PLCR
L15           5 S L14 AND (ANTIBOD? OR ANTIGEN? OR MONOCLONAL? OR ?IMMUNOA
SSA
L16           1 S L14 (P) (ANTIBOD? OR ANTIGEN? OR MONOCLONAL? OR ?IMMUNOA
SSA
L17           4 S L15 NOT L16
L18           3 S L1 (P) (ANTIBOD?) (P) (PLCR OR LIGASE CHAIN)
L19          12 S DIOLIGONUCLEOTIDE? OR DI-OLIGONUCLEOTIDE?
L20          12 S L19 AND LIGASE?
L21           2 S L19 (P) LIGASE
L22           2 S L20 AND IMMUNOASSAY?
L23           1 S L22 NOT L21
L24           0 S IMMUNO-PLCR
L25           0 S IMMUNOLCR
L26           0 S IMMUNO-LCR
L27           0 S IMMUNOLIGASE CHAIN REACTION
                E IMMUNOLIG
L28           1 S IMMUNOASSAY (2A) (LIGASE CHAIN OR LCR)
L29           1 S IMMUNOASSAY (5A) (LIGASE CHAIN OR LCR)
L30           0 S (ANTIBOD? OR MONOCLONAL?) (5A) (LIGASE CHAIN OR LCR)
L31           1 S (ANTIBOD? OR MONOCLONAL?) (10A) (LIGASE CHAIN OR LCR)
L32        1188 S LIGASE CHAIN REACTION? OR LCR OR PLCR
L33          52 S L32 (P) (ANTIBOD? OR MONOCLONAL? OR IMMUNOASSAY?)
L34           0 S L33 (P) EPITOPES
L35          32 S THREE EPITOPES
L36           0 S L32 AND L35
L37          15 S THIRD EPITOPE
L38           0 S L32 AND L37
L39         285 S (THREE OR THIRD) (3A) (EPITOPE? OR DETERMINANT?)
L40          21 S (L1 OR DI-OLIGONUCLEOTIDE? OR L32) (3P) L39
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APS search 6/98

(FILE 'USPAT' ENTERED AT 09:12:26 ON 15 JUN 1998)

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L1         26324 S PYRIDYL?
L2           438 S L1 (P) (ANTIBOD? OR MONOCLONAL? OR POLYCLONAL? OR IGG OR
 IM
L3             5 S L2 (3P) LIGAT?
L4           378 S L2 (3P) (CROSSLINK? OR CROSS-LINK? OR LINK? OR ATTACH? O
R C
L5            70 S L4 AND OLIGONUCLEO?
L6         97930 S CROSS-LINK? OR CROSSLINK?
L7           408 S L6 (P) OLIGONUCLEOT?
L8            16 S L1 (3P) L7
L9            12 S L8 AND (ANTIBOD? OR MONOCLONAL? OR POLYCLONAL? OR IMMUNO
GLO
L10           12 S L8 AND L9
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=> d cit fd 1-12

1. 5,744,326, Apr. 28, 1998, Use of viral CIS-acting post-transcriptional regulatory sequences to increase expression of intronless genes containing near-consensus splice sites; Charles R. Ill, et al., 435/172.3, 320.1; 536/24.1 [IMAGE AVAILABLE]

US PAT NO: 5,744,326 [IMAGE AVAILABLE] L10: 1 of 12
DATE FILED: Mar. 11, 1996

2. 5,728,518, Mar. 17, 1998, Antiviral poly-and oligonucleotides; Ellen Carmichael, 435/5, 6, 7.1; 536/23.1, 24.5 [IMAGE AVAILABLE]

US PAT NO: 5,728,518 [IMAGE AVAILABLE] L10: 2 of 12
DATE FILED: Aug. 8, 1994

3. 5,719,271, Feb. 17, 1998, Covalently cross-linked oligonucleotides; Phillip Dan Cook, et al., 536/23.1, 24.3, 24.5 [IMAGE AVAILABLE]

US PAT NO: 5,719,271 [IMAGE AVAILABLE] L10: 3 of 12
DATE FILED: Aug. 30, 1994

4. 5,700,642, Dec. 23, 1997, Oligonucleotide sizing using immobilized cleavable primers; Joseph Albert Monforte, et al., 435/6, 91.2 [IMAGE AVAILABLE]

US PAT NO: 5,700,642 [IMAGE AVAILABLE] L10: 4 of 12
DATE FILED: May 22, 1995

5. 5,656,731, Aug. 12, 1997, Nucleic acid-amplified **immunoassay** probes; Michael S. Urdea, 530/391.1; 435/6, 7.1, 7.7, 7.72, 91.1, 810; 436/501; 530/387.1, 391.3, 391.5; 536/23.1, 24.1, 24.31, 24.32, 24.33; 935/77, 78 [IMAGE AVAILABLE]

US PAT NO: 5,656,731 [IMAGE AVAILABLE] L10: 5 of 12
DATE FILED: Jul. 1, 1993

6. 5,648,218, Jul. 15, 1997, Preparation of photoprotein conjugates and methods of use thereof; Nancy L. Stults, 435/6, 5, 7.1, 7.2, 7.9; 536/24.3, 26.6 [IMAGE AVAILABLE]

US PAT NO: 5,648,218 [IMAGE AVAILABLE] L10: 6 of 12

DATE FILED: Jun. 6, 1995

7. 5,648,213, Jul. 15, 1997, Compositions and methods for use in detection of analytes; M. Parameswara Reddy, et al., 435/6, 7.1, 7.23; 436/538; 536/23.1 [IMAGE AVAILABLE]

US PAT NO: 5,648,213 [IMAGE AVAILABLE] L10: 7 of 12
DATE FILED: Aug. 30, 1994

8. 5,543,507, Aug. 6, 1996, Covalently cross-linked oligonucleotides; Phillip D. Cook, et al., 536/23.1; 435/91.1, 91.21, 91.4, 91.5; 536/24.1, 24.2, 24.3, 24.31, 24.32, 24.33, 25.2, 25.3 [IMAGE AVAILABLE]

US PAT NO: 5,543,507 [IMAGE AVAILABLE] L10: 8 of 12
DATE FILED: Mar. 2, 1994

9. 5,521,298, May 28, 1996, High specific activity nucleotide probes having target recognition and signal generation moieties; Chander Bahl, et al., 536/24.3; 435/6, 91.1, 91.2; 536/22.1, 23.1, 24.1, 24.31, 24.32, 24.33, 25.3; 935/77, 78 [IMAGE AVAILABLE]

US PAT NO: 5,521,298 [IMAGE AVAILABLE] L10: 9 of 12
DATE FILED: Oct. 25, 1993

10. 5,486,455, Jan. 23, 1996, Photoprotein conjugates and methods of use thereof; Nancy L. Stults, 435/6, 5, 7.1; 536/26.6 [IMAGE AVAILABLE]

US PAT NO: 5,486,455 [IMAGE AVAILABLE] L10: 10 of 12
DATE FILED: Aug. 22, 1994

11. 5,391,723, Feb. 21, 1995, Oligonucleotide conjugates; John H. Priest, 536/23.1; 530/402 [IMAGE AVAILABLE]

US PAT NO: 5,391,723 [IMAGE AVAILABLE] L10: 11 of 12
DATE FILED: Feb. 16, 1993

12. 5,324,829, Jun. 28, 1994, High specific activity nucleic acid probes having target recognition and signal generating moieties; Chander Bahl, et al., 536/23.1; 435/6; 536/22.1, 24.2, 25.3, 25.4; 935/17, 77, 78 [IMAGE AVAILABLE]

US PAT NO: 5,324,829 [IMAGE AVAILABLE] L10: 12 of 12
DATE FILED: Dec. 16, 1988

	5,744,326	[IMAGE AVAILABLE]
2.	5,728,518	[IMAGE AVAILABLE]
3.	5,719,271	[IMAGE AVAILABLE]
4.	5,700,642	[IMAGE AVAILABLE]
5.	5,656,731	[IMAGE AVAILABLE]
6.	5,648,218	[IMAGE AVAILABLE]
7.	5,648,213	[IMAGE AVAILABLE]
8.	5,543,507	[IMAGE AVAILABLE]
9.	5,521,298	[IMAGE AVAILABLE]
10.	5,486,455	[IMAGE AVAILABLE]
11.	5,391,723	[IMAGE AVAILABLE]
12.	5,324,829	

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#	Patent	Source	Flag	I	Date	Pages	Current Original Classif	Retrie[redacted] Classif	Current Cross Reference
1	5,693,764	U	T		12/02/1997	17	530/391.1		436/547 ...
2	5,670,381	U	T		09/23/1997	34	436/518		435/7.92 ...
3	5,650,284	U	T		07/22/1997	12	435/7.1		435/7.72 ...
4	5,639,656	U	T		06/17/1997	29	435/344.1		435/332 ...
5	5,573,921	U	T		11/12/1996	19	435/7.92		422/56 ...
6	5,496,700	U	T		03/05/1996	14	435/7.1		435/4 ...
7	5,459,080	U	T		10/17/1995	36	436/538		435/7.92 ...
8	5,399,501	U	T		03/21/1995	16	436/532		435/174 ...
9	5,149,626	U	T		09/22/1992	19	435/7.9		435/7.1 ...
10	5,147,786	U	T		09/15/1992	12	435/7.94		435/975 ...
11	5,026,653	U	T		06/25/1991	21	436/518		422/58 ...
12	4,748,111	U	T		05/31/1988	8	435/6		435/7.23 ...

EDS MAYA Classification Report for 981310.

1. 436/518 Total=25 ORs=7 XRs=18
   Class 436 CHEMISTRY: ANALYTICAL AND IMMUNOLOGICAL TESTING
   Sub 518 INVOLVING AN INSOLUBLE CARRIER FOR IMMOBILIZING IMMUNOCHEMICALS

2. 435/7.92 Total=18 ORs=5 XRs=13
   Class 435 CHEMISTRY: MOLECULAR BIOLOGY AND MICROBIOLOGY
   Sub 4 MEASURING OR TESTING PROCESS INVOLVING ENZYMES OR MICRO-ORGANISMS; COMPOSITION OR TEST STRIP THEREFORE; PROCESSES OF FORMING SUCH COMPOSITION OR TEST STRIP
   Sub 7.1 .Involving antigen-antibody binding, specific binding protein assay or specific ligand-receptor binding assay
   Sub 7.9 ..Assay in which an enzyme present is a label
   Sub 7.92 ...Heterogeneous or solid phase assay system (e.g., ELISA, etc.)

3. 435/7.94 Total=18 ORs=2 XRs=16
   Class 435 CHEMISTRY: MOLECULAR BIOLOGY AND MICROBIOLOGY
   Sub 4 MEASURING OR TESTING PROCESS INVOLVING ENZYMES OR MICRO-ORGANISMS; COMPOSITION OR TEST STRIP THEREFORE; PROCESSES OF FORMING SUCH COMPOSITION OR TEST STRIP
   Sub 7.1 .Involving antigen-antibody binding, specific binding protein assay or specific ligand-receptor binding assay
   Sub 7.9 ..Assay in which an enzyme present is a label
   Sub 7.92 ...Heterogeneous or solid phase assay system (e.g., ELISA, etc.)
   Sub 7.94 ....Sandwich assay

4. 435/7.1 Total=14 ORs=3 XRs=11
   Class 435 CHEMISTRY: MOLECULAR BIOLOGY AND MICROBIOLOGY
   Sub 4 MEASURING OR TESTING PROCESS INVOLVING ENZYMES OR MICRO-ORGANISMS; COMPOSITION OR TEST STRIP THEREFORE; PROCESSES OF FORMING SUCH COMPOSITION OR TEST STRIP
   Sub 7.1 .Involving antigen-antibody binding, specific binding protein assay or specific ligand-receptor binding assay

5. 435/7.93 Total=13 ORs=0 XRs=13
Class 435 CHEMISTRY: MOLECULAR BIOLOGY AND MICROBIOLOGY
Sub 4 MEASURING OR TESTING PROCESS INVOLVING ENZYMES OR MICRO-ORGANISMS; COMPOSITION OR TEST STRIP THEREFORE; PROCESSES OF FORMING SUCH COMPOSITION OR TEST STRIP
Sub 7.1 .Involving antigen-antibody binding, specific binding protein assay or specific ligand-receptor binding assay
Sub 7.9 ..Assay in which an enzyme present is a label
Sub 7.92 ...Heterogeneous or solid phase assay system (e.g., ELISA, etc.)
Sub 7.93 ....Competitive assay

6. 435/6 Total=12 ORs=7 XRs=5
Class 435 CHEMISTRY: MOLECULAR BIOLOGY AND MICROBIOLOGY
Sub 4 MEASURING OR TESTING PROCESS INVOLVING ENZYMES OR MICRO-ORGANISMS; COMPOSITION OR TEST STRIP THEREFORE; PROCESSES OF FORMING SUCH COMPOSITION OR TEST STRIP
Sub 6 .Involving nucleic acid

7. 435/5 Total=11 ORs=9 XRs=2
Class 435 CHEMISTRY: MOLECULAR BIOLOGY AND MICROBIOLOGY
Sub 4 MEASURING OR TESTING PROCESS INVOLVING ENZYMES OR MICRO-ORGANISMS; COMPOSITION OR TEST STRIP THEREFORE; PROCESSES OF FORMING SUCH COMPOSITION OR TEST STRIP
Sub 5 .Involving virus or bacteriophage

8. 435/975 Total=11 ORs=0 XRs=11
Class 435 CHEMISTRY: MOLECULAR BIOLOGY AND MICROBIOLOGY
Sub 975 KIT

9. 436/531 Total=10 ORs=0 XRs=10
Class 436 CHEMISTRY: ANALYTICAL AND IMMUNOLOGICAL TESTING
Sub 518 INVOLVING AN INSOLUBLE CARRIER FOR IMMOBILIZING IMMUNOCHEMICALS
Sub 528 .Carrier is organic
Sub 531 ..Carrier is synthetic resin

10. 435/7.9 Total=9 ORs=1 XRs=8
Class 435 CHEMISTRY: MOLECULAR BIOLOGY AND MICROBIOLOGY
Sub 4 MEASURING OR TESTING PROCESS INVOLVING ENZYMES OR MICRO-ORGANISMS; COMPOSITION OR TEST STRIP

THEREFORE; PROCESSES OF FORMING SUCH COMPOSITION OR TEST STRIP

Sub 7.1 .Involving antigen-antibody binding, specific binding protein assay or specific ligand-receptor binding assay

Sub 7.9 ..Assay in which an enzyme present is a label

11. 435/810 Total=9 ORs=0 XRs=9
Class 435 CHEMISTRY: MOLECULAR BIOLOGY AND MICROBIOLOGY
Sub 810 PACKAGED DEVICE OR KIT

12. 436/501 Total=9 ORs=0 XRs=9
Class 436 CHEMISTRY: ANALYTICAL AND IMMUNOLOGICAL TESTING
Sub 501 BIOSPECIFIC LIGAND BINDING ASSAY

(FILE 'USPAT' ENTERED AT 09:12:26 ON 15 JUN 1998)
DELETE HISTORY Y

L1 13 SEARCH (5561049 OR 5254458 OR 5026653 OR 4748111 OR 446978
7 O
L2 13 SEARCH (5459078 OR 5670381 OR 5424219 OR 4514508 OR 469089
0 O
L3 13 SEARCH (4376110 OR 5364760 OR 5656731 OR 5391272 OR 554105
9 O
L4 11 SEARCH (4486530 OR 4468470 OR 5580730 OR 5650284 OR 450314
3 O
L5 50 SEARCH L1 OR L2 OR L3 OR L4
L6 8 S L5 AND PYRIDYL?
L7 8 S L6 AND (ANTIBOD? OR MONOCLONAL? OR POLYCLONAL? OR IMMUNO
GLO

=> d cit 1-8

1. **5,670,381**, Sep. 23, 1997, Devices for performing ion-capture binding assays; Yi-Her Jou, et al., 436/518; 435/7.92, 7.93, 7.94, 7.95, 970, 971; 436/529, 536, 538, 540, 541, 808, 810 [IMAGE AVAILABLE]

2. **5,656,731**, Aug. 12, 1997, Nucleic acid-amplified **immunoassay** probes; Michael S. Urdea, 530/391.1; 435/6, 7.1, 7.7, 7.72, 91.1, 810; 436/501; 530/387.1, 391.3, 391.5; 536/23.1, 24.1, 24.31, 24.32, 24.33; 935/77, 78 [IMAGE AVAILABLE]

3. **5,639,656**, Jun. 17, 1997, **Antibodies** reactive with biological markers of benign prostate hyperplasia; George L. Wright, Jr., 435/344.1, 332; 530/388.2, 388.85, 389.1, 391.1, 391.3, 391.7 [IMAGE AVAILABLE]

4. **5,593,825**, Jan. 14, 1997, Hepatitis B virus mutants, reagents and methods for detection; William F. Carman, et al., 435/5, 69.3, 252.3, 254.11, 320.1, 325, 352, 362, 367, 975; 536/23.72, 24.32 [IMAGE AVAILABLE]

5. **5,459,080**, Oct. 17, 1995, Ion-capture assays using a specific binding member conjugated to carboxymethylamylose; Janina Adamczyk, et al., 436/538; 435/7.92, 7.93, 7.94, 961; 436/528, 539, 541, 544 [IMAGE AVAILABLE]

6. **5,459,078**, Oct. 17, 1995, Methods and reagents for performing ion-capture digoxin assays; Steven Kline, et al., 436/518; 435/6, 7.92, 7.93, 7.94, 7.95, 970, 971, 975; 436/529, 536, 538, 540, 541, 808, 810, 824, 825 [IMAGE AVAILABLE]

7. **5,399,501**, Mar. 21, 1995, Covalent attachment of specific binding members to a solid phase with two bifunctional reagents and a dithio compound; Mark R. Pope, et al., 436/532; 435/174, 181, 974; 436/518, 811; 530/810, 816 [IMAGE AVAILABLE]

8. **4,690,890**, Sep. 1, 1987, Process for simultaneously detecting multiple antigens using dual sandwich immunometric assay; Rueyming Loor, et al., 435/7.21, 7.32, 7.4, 7.94, 21, 172.2, 810, 948, 969, 975; 436/531, 533, 548, 808, 813, 815, 819; 935/89, 110 [IMAGE AVAILABLE]

Automation; Gene library; Polymerase chain reaction; ELISA assay; Sensitivity; Specificity; Yeast artificial chromosome

French Descriptors: DNA chromosomique; Clonage moleculaire; Criblage; Methode; Automatisation; Banque gene; Reaction chaine polymerase; Technique ELISA; Sensibilite; Specificite; Chromosome artificiel levure

Classification Codes: 002A31C02A4; 215

5/9/10 (Item 1 from file: 173)
DIALOG(R)File 173:Adis LMS Drug Alerts

00621213	800637528
TITLE:	**Oligonucleotide ligation** assay for detecting mutations in the human immunodeficiency virus type 1 pol gene that are associated with resistance to zidovudine, didanosine, and lamivudine.
AUTHOR:	Edelstein R E; **Nickerson D A**; Tobe V O; et al
JOURNAL:	Journal of Clinical Microbiology (J-Clin-Microbiol) 36: 569-572, Feb 1998.
PUBLICATION DATE:	1 February 1998 (19980201)
LANGUAGE:	English
ADIS LMS:	Antivirals (Index only): Alert no. 3, 1998
RECORD TYPE:	Citation
DOCUMENT TYPE:	In vitro
DESCRIPTORS:	Antimicrobial-resistance; Clinical-isolates; Didanosine, antimicrobial-activity; HIV-1-infections; Lamivudine, antimicrobial-activity; Zidovudine, antimicrobial-activity

80 bases in size are synthesized as fragments and **ligated** together. After generation, probes are labeled at the 3' terminus with rhodamine. All the probes are synthesized with a 5' biotin and these biotinylated probes are attached to the bottom of a plate coated with immobilized streptavidin. Probes are attached along a 10.times.10 array and ordered according to size (FIG. 5B).

US PAT NO: 5,635,602 [IMAGE AVAILABLE] L6: 2 of 3

ABSTRACT:
The invention relates to bis-protein-DNA **conjugates**. A protein having an antigen specific binding activity is covalently linked to each end of a derivatized DNA molecule. The bis-protein-DNA **conjugates** can be used for immunoassays and measuring distances between proteins at up to 3.4 .ANG. resolution. The invention also relates to methods of synthesizing these bis-protein-DNA **conjugates**. Synthesis of the **conjugates** entails derivatizing the 5' or 3' end of a DNA **oligonucleotide** and covalently linking that DNA to a protein. The DNA can be indirectly **conjugated** to an antibody or Fab' fragment, using a avidin/streptavidin-biotin linkage. The **conjugates** of the invention can be used in **immunoassays** and **PCR** assays.

DETDESC:

DETD(114)

Ever since the elucidation of the double helical structure of DNA, its use as genetic material has been widely observed, utilized and exploited. However, to date, the utility of the DNA molecule as a non-genetic chemical has yet to be fully realized. This Example shows that end-derivatized double-stranded DNA can serve as a cross-linker between different immunoglobulin G Fab' fragments to form a semisynthetic bispecific antibody. Beyond its function as a novel cross-linker, though, DNA possesses a number of potentially very useful properties, including the following: gentle hetero-cross-linking via simple annealing reactions; automated synthesis; controllable sequence variation resulting in well-defined length and structure (useful up to hundreds of base pairs); susceptibility to intercalator incorporation resulting in controllable length, symmetry and stiffness; localized and reversible scission via restriction/religation; and exquisitely sensitive detection via the polymerase chain reaction. However, to enable enzymatic action by restriction enzymes against the intact cross-linking DNA molecule, a somewhat longer DNA sequence should be used as the 32mer (SEQ ID NOS: 1 and 2) sequence used here was largely resistant to cleavage by all of the restriction enzymes for which the 32mer oligonucleotide (SEQ ID NOS: 1 and 2) has known sites (data not shown). The unconjugated 32mer sequence (SEQ ID NOS: 1 and 2), on the other hand, was susceptible to cleavage by these restriction enzymes, as expected (data not shown). Likely explanations for this behavior are steric hindrance or altered DNA structure. Another difficulty is that reducing conditions are necessary for many DNA modifying enzymes, including **ligases** and many restriction enzymes. Unfortunately, the model constructs described in this paper all contain easily usable disulfide bond cross-links between protein and DNA. Treatment with reducing agent will destroy these important structural bonds. For applications that require irreversiblity of the protein-DNA cross-link, an irreversible thioether bond may be produced (Glennie et al., 1987).

DETDESC:

DETD(148)

In order to follow changes in energy transfer efficiency, we define E'(t)=E(t)/E.sub.0, where E(t) is the energy transfer efficiency per cell obtained for HPB-ALL following modulation at 37.degree. C. for t min and E.sub.0 is the energy transfer efficiency per cell obtained for unmodulated cells. E is inversely proportional to the distance separating donor and acceptor (Eq. 3). However, it is essential to bear in mind that, on a flow system, fluorescence intensities are detected as signals integrated over the entire cell, such that F.sub.STRITC is a function of n.sub.D+A, the number of donor- and acceptor-labeled assemblies per cell and F.sub.FITC is a function of n.sub.D, the number of donor-labeled epitopes per cell. When n.sub.D+A .noteq.n.sub.D and n.sub.D+A /n.sub.D .noteq.k, it is often impossible to differentiate between changes in proximity and changes in the number of assemblies relative to the number of donor-labeled epitopes on the cell surface. Therefore, it is more appropriate to interpret changes in E' as changes in CD4-TCR association following CD4 **ligation** at 0.degree. C. and 37.degree. C.

DETDESC:

DETD(172)

A restriction endonuclease corresponding to the restriction enzyme recognition site present in the DNA is applied to each well after the addition of an appropriate reaction solution for the restriction endonuclease, and allowed to digest the linker DNA molecule of the bis(Fab')-DNA conjugate. After the digestion, the microtiter plate is washed extensively with ETBS. When the conjugate binds either Ag-1 or Ag-2, one of the Fab' fragments with a half of the digested linker DNA is removed by washing. Both Fab' fragments remain bound to antigens only when the conjugate binds both Ag-1 and Ag-2 simultaneously. Then, DNA **ligase** is added to the wells to religate the digested linker DNA, after the addition of an appropriate **ligation** solution to wells. When a bis(Fab')-DNA conjugate binds both Ag-1 and Ag-2, religation of the linker DNA occurs. However, when one of the paired Fab' fragments is removed, no **ligation** reaction would occur because of the absence of the other half of the linker DNA. Then, PCR is performed on the microtiter plate wells under standard conditions to amplify the religated linker DNA. A set of primers, which hybridize to the linker DNA flanking the restriction enzyme recognition sequence, are used. The resulting PCR product is analyzed by an appropriate method, such as gel electrophoresis. The PCR product can be produced only when the bis(Fab')-DNA conjugate binds two different antigens simultaneously and the linker DNA is religated. Thus, the presence of the specific PCR product demonstrates that two antigens, Ag-1 and Ag-2, are present in the sample. Further, this method cuts down on the noise and background in immuno-PCR. When only one antigen is present in the sample, a shorter PCR product, which corresponds to the length from one of the primer-annealing site to the restriction endonuclease recognition site is produced. However, such PCR products can be discriminated from the full-length PCR product produced from the religated linker DNA. In addition, the amount of such PCR products is much smaller than the full-length PCR product, because one Fab' fragment with a half of the linker DNA allows only linear PCR amplification of a portion of the linker DNA, not the exponential PCR amplification which occurs with the religated linker DNA.

DETDESC:

DETD(214)
Mittler, R. S., Goldman, S. J., Spitalny, G. L., and Burakoff, S. J. (1989a). T-cell receptor CD4 physical association in a murine T-cell hybridoma: induction by antigen receptor **ligation**. Proc. Natl. Acad. Sci. USA 86, 8531-8535.

US PAT NO: 5,384,255 [IMAGE AVAILABLE] L6: 3 of 3

SUMMARY:

BSUM(7)

Ubiquitin-mediated protein degradation is a multi-step process. Briefly, **ligation** of ubiquitin is initiated by the activation of its C-terminal Gly residue, which is catalyzed by a specific ubiquitin-activating enzyme, E1. This step consists of the intermediary formation of ubiquitin adenylate (with the displacement of PPi from ATP), and the transfer of activated ubiquitin to a thiol site of E1 (with the subsequent release of AMP). Next, activated ubiquitin is transferred by transacylation to thiol groups of a family of ubiquitin-carrier proteins, E2(s). E2-ubiquitin thiol esters are the donors of ubiquitin for isopeptide bond formation between the C-terminal Gly residue of ubiquitin and .epsilon.-amino groups of Lys residues of proteins. Ubiquitin-protein **ligation** may occur by direct transfer from E2, or by a process in which target proteins are first bound to specific sites of ubiquitin-protein **ligases**, E3(s), and then ubiquitin is transferred from E2. Proteins which are **ligated** to multiple ubiquitin units are degraded by a 26S protease complex.

SUMMARY:

BSUM(11)

Two species of ubiquitin-protein **ligases**, E3s, have been purified and characterized. One enzyme, E3.alpha., binds noncovalently to ubiquitin via recognition of complementary structural motifs (Hershko, A. et al., J. Biol. Chem. 258:8206-8214 (1983)). This **ligase** recognizes mostly proteins with basic (Arg, His, Lys) and bulky-hydrophobic (Leu, Phe, Trp, Tyr) free N-terminal residues (Reiss, Y. et al., J. Biol. Chem. 263:2693-2698 (1988); Reiss, Y. et al., J. Biol. Chem. 265:3685-3690 (1990); Reiss, Y. et al., J. Biol. Chem. 264:10378-10383 (1989)). The second **ligase** characterized, E3.beta., recognizes proteins with small uncharged residues (such as Ala, Ser, and Thr) at their N-terminal residue (Heller, H. et al., J. Biol. Chem. 265:6532-6535 (1990)), though penultimate amino acid residues also contribute to the recognition (H. Heller and A. Hershko, unpublished results).

SUMMARY:

BSUM(16)

It is assumed that the E2 enzymes involved in proteolysis recognize distinct species of E3s. However, it is not known whether they also have specific recognition sites for the substrates. This task is accomplished, most probably, by the different **ligases**. Of the five E2s isolated originally from reticulocytes, only the 14 kDa enzyme was shown to be involved in E3-dependent multiple ubiquitination and subsequent degradation (Pickart, C. M. et al., J. Biol. Chem. 260:1573-1581 (1985)).

Initial analysis of the mechanism of action of E3.alpha. revealed that in addition to the specific binding sites for ubiquitin and the substrate, it also interacts with one (or more) of the E2 enzymes with which it acts in concert (Reiss, Y. et al., J. Biol. Chem. 265:3685-3690 (1990); Reiss, Y. et al., J. Biol. Chem. 264:10378-10383 (1989)).

SUMMARY:

BSUM(17)

The formation of an E2-E3 complex probably facilitates the transfer of activated ubiquitin from E2 to the protein substrate bound to the **ligase**. Successive studies have shown that the ability of UBC3 (RAD6) to promote polyubiquitination and degradation in vitro is dependent upon the presence of E3.alpha. (Sung, P. et al., EMBO J. 10:2187-2193 (1991)). A similar study carried out in yeast demonstrated that UBC2 is physically associated with UBR1, the yeast homolog of E3.alpha. that recognizes certain proteins via their free N-terminal residues; immunoprecipitation of UBR1 also precipitated UBC2 (Jurgen Dohmem, R. et al., Proc. Natl. Acad. Sci. USA 88:7351-7355 (1991)). In addition, certain UBR1 substrates ("N-end rule" substrates; Varshavsky, A., Cell 69:725-735 (1992)) such as Arg-.beta.gal and Leu-.beta.gal were dramatically stabilized in UBC2 null mutants (Jurgen Dohmem, R. et al., Proc. Natl. Acad. Sci. USA 88:7351-7355 (1991); Varshavsky, A., Cell 69:725-735 (1992)).

SUMMARY:

BSUM(19)

Thus, if the notion that different E2 enzymes interact with specific **ligases** is true, a reasonable conclusion is that the "N-end" pathway E2s (the yeast UBC2 and the mammalian E2-14kDa) do not play an important role in ubiquitin-mediated proteolysis. Therefore, other, yet unidentified species of E2(s) probably account for targeting of most cellular proteins for degradation. One such E2 has been recently purified and characterized from wheat germ (Girod, P.-A. et al., J. Biol. Chem. 268:955-960 (1993)).

SUMMARY:

BSUM(54)

The present invention is also directed to a method of using the E2-F1 enzyme to detect the presence of E3 protein **ligase** in a biological sample, comprising the steps of:

SUMMARY:

BSUM(58)
(a) incubating the substance with E2-F1, E1, ATP, ubiquitin, a source of E3 **ligase**, and a substrate protein, whereby tile incubation promotes the conjugation of ubiquitin with the protein substrate;

DETDESC:

DETD(21)

Purified E2-F1 can also be used to identify an E3 protein **ligase** in a biological sample. For example, E3 can be identified in a biological

fluid or cellular extract by determining whether the biological sample promotes the formation of ubiquitin-protein conjugates in the presence of E1 and purified E2-F1 (see Example 3). In addition, purified E2-F1 may be used to construct an E2-F1 affinity column, using well known techniques (for example, see Affinity Chromatography: A Practical Approach, Dean et al. (Eds.) IRL Press, Washington, D.C. (1985)). Such an E2-F1 affinity column may be used to bind E3 enzyme from a biological sample, as a first step in the isolation and purification of E3 enzyme.

DETDESC:

DETD(62)

Alternatively, antibodies specific for E2-F1, or a functional derivative, may be delectably labeled with DNA by the technique of immuno-polymerase chain reaction (Sano et at., Science 258:120-122 (1992)). The polymerase chain reaction (PCR) procedure amplifies specific nucleic acid sequences through a series of manipulations including denaturation, annealing of oligonucleotide primers, and extension of the primers with DNA polymerase (see, for example, Mullis et al., U.S. Pat. No. 4,683,202; Mullis et al., U.S. Pat. No. 4,683,195; Loh et al., Science 243:217 (1988)). The steps can be repeated many times, resulting in a large amplification of the number of copies of the original specific sequence. As little as a single copy of a DNA sequence can be amplified to produce hundreds of nanograms of product (Li et al., Nature 335:414 (1988)). Other known nucleic acid amplification procedures include transcription-based amplification systems (Kwoh et al., Proc. Natl. Acad. Sci. USA 86:1173 (1989); Gingeras et al., WO 88/10315), and the "**ligase** chain reaction" in which two (or more) oligonucleotides are **ligated** in the presence of a nucleic acid target having the sequence of the resulting "di-oligonucleotide" thereby amplifying the di-oligonucleotide (Wu et al., Genomics 4:560 (1989); Backman et al., EP 320,308; Wallace, EP 336,731; Orgel, WO 89/09835).

DETDESC:

DETD(63)

For example, the **immuno-PCR** assay can be carried out by immobilizing various amounts of the test material on the surface of microtiter wells (see Sanzo et al., supra, page 122, footnote 7). The wells are subsequently incubated with E2-F1 monoclonal antibody, washed, and then incubated with biotinylated DNA molecules which have been **conjugated** to streptavidin-protein chimera (Id.). This chimera binds biotin (via the streptavidin moiety) and the Fc portion of an immunoglobulin G molecule (via the protein A moiety) (Id., at 120; Sanzo et al., Bio/Technology 9:1378 (1991)). (Alternatively, a commercially available avidin system may be used instead of the chimera, as described by Ruzicka et al., Science 260:698-699 (1993)). The wells are then washed to remove unbound **conjugates**. Any E2-F1 present in the test material will be bound by the E2-F1 monoclonal antibody, which ill turn, is bound by tile protein A moiety of the biotinylated DNA-streptavidin-protein A **conjugate**. Then, the DNA sequences are amplified using PCR. Briefly, the microtiter wells are incubated with deoxyribonucleoside triphosphates, **oligonucleotide** primers, and Taq DNA polymerase (see Sanzo et al., supra, page 122, footnote 11). An automated thermal cycler (such as the PTC-100-96 Thermal Cycler, MJ Research, Inc.) can be used to perform PCR under standard conditions (Id.). The PCR products are then analyzed by agarose gel electrophoresis after staining with ethidium

bromide.

DETDESC:

DETD(80)

For cloning into a vector, suitable DNA preparations (either genomic or cDNA) are randomly sheared or enzymatically cleaved, respectively, and **ligated** into appropriate vectors to form a recombinant gene (either genomic or cDNA) library. A DNA sequence encoding E2-F1 may be inserted into a vector in accordance with conventional techniques, including blunt-ending or staggered-ending termini for **ligation**, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and **ligation** with appropriate **ligases**. Techniques for such manipulation are disclosed by Sambrook et al., supra, and are well known in the art.

DETDESC:

DETD(115)

Thus, crude reticulocyte Fraction 1 stimulates the degradation of GA3PDH. This dehydrogenase is a homotetramer that is composed of four 35 kDa molecular mass chains with a Val residue in their N-terminal position. Val is a "stabilizing" residue according to the N-end rule in bacteria, yeast, and mammalian cells (Varshavsky, A., Cell 69:725-735 (1992)). It was clear, therefore, that this protein is not recognized by E3.alpha. and E3.beta., the two ubiquitin-protein **ligases** that recognize certain "destabilizing" residues in N-terminal free proteins.

11. 5,635,602, Jun. 3, 1997, Design and synthesis of bispecific DNA-antibody conjugates; Charles R. Cantor, et al., 530/391.1, 387.3, 391.5, 391.9; 536/23.1 [IMAGE AVAILABLE]

US PAT NO: 5,635,602 [IMAGE AVAILABLE] L2: 11 of 18

ABSTRACT:
The invention relates to bis-protein-DNA conjugates. A protein having an antigen specific binding activity is covalently linked to each end of a derivatized DNA molecule. The bis-protein-DNA conjugates can be used for immunoassays and measuring distances between proteins at up to 3.4 .ANG. resolution. The invention also relates to methods of synthesizing these bis-protein-DNA conjugates. Synthesis of the conjugates entails derivatizing the 5' or 3' end of a DNA oligonucleotide and covalently linking that DNA to a protein. The DNA can be indirectly conjugated to an antibody or Fab' fragment, using a avidin/streptavidin-biotin linkage. The conjugates of the invention can be used in **immunoassays** and **PCR** assays.